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THE NUTRITION OF GREAT LAKES CLADOPHORA

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A synthetic culture medium was developed for the laboratory culture of Cladophora glomerata from the Great Lakes. Recognition that Cladophora requires vitamins B_1 and B_{12} was a key feature in the successful culture of this alga. Systematic modifications of the initial culture solution resulted in an optimum culture medium in which C. glomerata routinely produced 500 mg oven-dry algae in a 21-day period.

The quantitative requirements of C. glomerata for essential inorganic nutrients were expressed primarily as critical cell concentrations, that is minimum cell concentrations which permit maximum yield. C. glomerata has relatively low critical N and P concentrations (1.1% and 0.06%, respectively) but very high B (110 ppm) and S (0.15%) critical concentrations. Ortho-, pyro-, meta, and tripoly-P can be utilized in C. glomerata growth as can NH_4 and NO_3-N. Phosphite-P is relatively unavailable.

Nutrient supplies and limiting nutrients for Cladophora sp. growth in the Great Lakes were evaluated by two bioassays, primarily plant analysis and to some extent the Fitzgerald tests. Plant analysis assays for P showed that Cladophora P concentrations correlated closely with the recognized pollution of areas sampled. Furthermore, P was limiting or close to limiting in several relatively unpolluted areas. There were indications that elements other than P at times limited Cladophora growth.

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THE NUTRITION OF GREAT LAKES CLADOPHORA

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ABSTRACT

A synthetic culture medium was developed for the laboratory culture of Great Lakes *Cladophora* sp. In initial phases of the study, *Cladophora* could be maintained in culture only when a sewage effluent or lake water supplement was added to the medium. With recognition that *Cladophora* has an unusual requirement, among freshwater green algae, for vitamins B_1 and B_{12}, the effluent or lake water supplement could be omitted from the culture medium. *Cladophora* was isolated from each of the Great Lakes with most of the detailed nutritional studies carried out on an isolate from Lake Michigan identified as *Cladophora glomerata*.

Systematic manipulations and modifications of the components of the initial culture medium led to considerable yield increase in a specific time interval. In the optimum culture medium that evolved, *C. glomerata* routinely produced 500 mg oven-dry algae in a 21-day growth period.

The quantitative requirements of *C. glomerata* for essential inorganic nutrients were expressed primarily as critical cell concentrations, that is minimum cell concentrations which permit maximum yield. Critical concentrations were established for most of the essential elements. *C. glomerata* can be characterized as having relatively low critical N and P concentrations (1.1% and 0.06%, respectively) but very high boron (110 ppm) and sulfur (.150%) critical concentrations. Ortho-, pyro-, meta, and tripoly-P can be utilized in the growth of *C. glomerata* as can NH_4 and NO_3-N. Phosphite-P is relatively unavailable.

Nutrient supplies and limiting nutrients for the growth of *Cladophora* sp. in the Great Lakes were evaluated by two plant bioassays, primarily plant analysis and to some extent the Fitzgerald tests. The plant analysis bioassay evaluates available nutrient supplies by comparisons of essential element concentrations in Great Lakes *Cladophora* samples and critical concentrations established in laboratory studies. Plant analysis assays for phosphorus in each of the Great Lakes showed that phosphorus concentrations in *Cladophora* correlated closely with the recognized pollution of the areas sampled. Furthermore, the assays indicated phosphorus to be limiting or close to limiting in several relatively unpolluted areas. Nevertheless, there were indications that elements other than phosphorus, particularly boron and to some extent zinc, at times limited *Cladophora* growth. There was some evidence that vitamin B_1 supply might limit *Cladophora* growth in the Great Lakes.

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SECTION I

CONCLUSIONS

There were two primary aspects to these studies on *Cladophora* sp., a primary nuisance green alga in the Great Lakes. One was concerned with developing suitable procedures for the laboratory culture of *Cladophora* and establishing its qualitative and quantitative nutrient requirements. The second aspect involved evaluations of nutritional factors that are critical in controlling growth of *Cladophora* in the Great Lakes.

The primary conclusions for this project can be summarized as follows:

1. Unlike most other algae found in midwestern lakes, *Cladophora glomerata* must be provided with external supplies of vitamins B_1 and B_{12}. It appears that *C. glomerata* is unable to synthesize required amounts of these vitamins.

2. With vitamins B_1 and B_{12} added to the culture medium, *C. glomerata* can be satisfactorily cultured in a completely defined medium and under environmental conditions similar to those used in the culture of other freshwater algae. Techniques now are available which make laboratory experimentation with *Cladophora* sp. relatively simple and easy.

3. When evaluated as critical cell concentrations (minimum concentrations which permit optimum growth), *C. glomerata* is indicated to have several unusual nutrient requirements. Compared to other freshwater algae, the requirements for nitrogen and phosphorus are relatively low. In comparison with plants in general, *C. glomerata* has very high requirements for boron and sulfur. Supplies of these elements in relation to *C. glomerata* requirements should be carefully considered in evaluating the nutrition of the alga in the Great Lakes.

4. Various forms of phosphorus (ortho-, pyro-, meta-, and tripoly-P) can be utilized by *Cladophora* sp., phosphite-P is relatively unavailable. *Cladophora* sp. can utilize both NH_4 and NO_3-N for growth.

5. Phosphorus concentrations in *Cladophora* from all of the Great Lakes correlate well with the recognized pollution of specific areas. Furthermore,

plant analysis bioassays indicate that phosphorus supply is limiting, or close to limiting, in areas with relatively little pollution. Although more data are needed, reduction in phosphorus supply probably is a practical means of reducing nuisance growths of Cladophora in the Great Lakes.

6. Nitrogen supply does not seem to limit Cladophora growth in the Great Lakes.

7. Bioassays by plant analysis indicate that supplies of elements other than phosphorus at times become limiting for Cladophora growth in the Great Lakes. This is particularly true for the trace element boron and perhaps for zinc. The possibility that vitamin B_1 supply limits Cladophora growth in the Great Lakes should not be disregarded and needs further study.

8. Plant bioassays (both plant analysis and the Fitzgerald tests) are recommended for evaluating nutrient supplies and growth-limiting nutrients for Cladophora in natural environments.

9. The sampling and analytical procedures used in the studies reported were sufficiently reliable so that in plant analysis bioassays significance could be attached to differences in nutrient concentrations in lake samples of Cladophora.

SECTION II
RECOMMENDATIONS

The development of plans and measures for the control of nuisance *Cladophora* sp. growths in the Great Lakes will be aided by (1) knowledge of the qualitative and quantitative nutritional requirements of the organisms, (2) recognition of unusual nutritional features of the *Cladophora* sp., and (3) the availability of simple procedures for accurate evaluation of nutrient supplies and growth-limiting nutrients in the lakes and streams where *Cladophora* occurs. This project has contributed to all of these requirements. The primary recommendations for the use and further development of the information obtained are:

1. As a result of the development of satisfactory procedures for the culture of *Cladophora* sp. in the laboratory, additional and confirmatory laboratory experiments on the qualitative and quantitative nutritional requirements of the *Cladophora* should be carried out.

2. Plant bioassays, demonstrated to be relatively simple and useful assay procedures, should be used in further tests of nutrient supplies and growth-limiting nutrients in the Great Lakes, particularly in areas known to vary markedly in degree of pollution.

3. The plant analysis bioassay should be further developed and refined for use with *Cladophora*. This should include more precise definitions of trace element critical cell concentrations, studies of factors which may cause critical concentration values to vary, and comparisons of the results of nutrient assays by plant analysis and other commonly used assay procedures.

4. Because of indications in the present study that phosphorus supplies were limiting or close to limiting in several Great Lakes areas from which *Cladophora* was collected, emphasis in further studies probably should be in clarifying the role of phosphorus as a growth-limiting element. Nevertheless, the possibility that nutrients other than phosphorus, both essential inorganic elements and vitamins, can become critical in nuisance *Cladophora* growths should be recognized and tested for.

5. The aggressiveness and effectiveness of Cladophora in competing for and absorbing nutrients at lake water concentrations should be established and compared with comparable data for other organisms with which Cladophora grows and competes in the Great Lakes.

6. The data and information from this and other similar studies should be utilized in developing plans for reducing nuisance growths of Cladophora in the Great Lakes.

SECTION III

INTRODUCTION

Green algae in the genus Cladophora are responsible for some of the most troublesome nuisance plant growths in the Great Lakes. Eutrophication and pollution have increased these nuisance growths. Identification of specific pollution factors which are primarily responsible for nuisance Cladophora growths and the development of plans for reducing these conditions should be based on data on the quantitative and qualitative nutritional requirements of the Cladophora.

The organisms most abundant in freshwater environments might be expected to have simple nutritional requirements and to be easy to culture in the laboratory. This has not been true. Bloom-producing blue-green algae, for example, were relatively difficult to bring into laboratory culture (Gerloff, Fitzgerald, and Skoog, 1950). In spite of their abundance in certain lakes, Cladophora sp. also have been difficult to culture in synthetic media. As a result, their specific nutritional requirements have been poorly understood.

The primary objectives of this study were (1) to culture nuisance Cladophora sp. from the Great Lakes in defined nutrient media, (2) to study under laboratory conditions the qualitative and quantitative nutritional requirements for optimum Cladophora sp. growth and (3) to evaluate the nutrition of Cladophora sp. in the Great Lakes and determine nutritional factors critical in controlling the growth of the algae under field conditions.

Requirements for both organic and inorganic factors must be determined when developing a culture medium for a specific alga and in evaluating its nutrition. As with other chlorophyll-containing organisms, most freshwater algae do not require external supplies of organic growth factors such as vitamins and amino acids. They are able to synthesize needed quantities of these compounds.

Both the qualitative and quantitative requirements of an alga for various mineral nutrients must be established. The qualitative nutrient requirements are concerned with the essentiality of specific elements for an organism. For normal growth, most plants require the 16 chemical elements routinely made available in synthetic culture media. A number of algae have unusual, specific additional requirements. For example, blue-green algae require sodium and cobalt, the green alga Scenedesmus quadricauda requires vanadium, and diatoms grow only in the presence of silicon. Quantitative inorganic nutrient requirements are concerned with the specific amounts and the balance of various elements necessary for optimum organism growth.

The successful culture of an alga requires consideration of other nutrient solution factors, such as pH and the total salt concentration. Optimum growth also usually occurs only within a narrow range of temperature and light with the optima differing for various organisms.

The quantitative requirements of an organism for essential inorganic nutrients can be expressed in several ways. Probably the most common expression is in terms of solution concentrations. These are by no means specific, and therefore are of limited value, because the volume of solution to which an organism is exposed also is a factor determining the total amount of each element available to an organism. The requirement for elements also can be expressed in terms of the critical concentrations, that is the minimum concentration of each element in a plant or plant part necessary for maximum yield, a concept discussed by Gerloff and Krombholz (1966), Gerloff (1969), and Gerloff and Fishbeck (1973). One of the primary goals of this project was to establish the critical concentration in Cladophora of all essential chemical elements likely to limit growth in the Great Lakes.

Evaluation of nutrient supplies and of the most likely growth-limiting nutrients in natural environments can be made by chemical analyses of water samples or by various bioassays. Several suggested advantages of the bioassay approach (Gerloff, 1969) were responsible for the emphasis of bioassays in evaluating the nutrition of Cladophora under field conditions on this project. One type of bioassay used is known as plant analysis. It involves comparison of the concentrations of key elements in field collected Cladophora sp. with the critical concentrations of those elements established in the laboratory. A nonlimiting supply of an element at sampling time is indicated if the concentration in an algae field sample is above the critical concentration; a concentration of the element at or below the critical concentration indicates the element had become growth-limiting in the environment (Gerloff and Krombholz, 1966; Gerloff, 1973). The second type of bioassay used is based on the rate of NH_4-N by algae in the dark as a measure of N supply and on the extraction of P from the algae as a measure of P availability (Fitzgerald, 1969).

SECTION IV

ISOLATION AND INITIAL CULTURE OF GREAT LAKES *Cladophora* sp.

To isolate Great Lakes *Cladophora* sp. for laboratory nutritional studies, it was necessary to have available a culture medium in which the algae would grow, or at least could be maintained. Unpurified *Cladophora* did not grow well in a medium generally satisfactory in the culture of many green algae, blue-green algae, and diatoms. This was a solution containing the concentrations of major essential elements specified by Hughes, Gorham, and Zehnder (1958) in a solution hereafter referred to as Gorham's Medium, and one-tenth the concentrations of trace elements in a culture solution developed by Johnson, Stout, Broyer, and Carlton (1957) hereafter referred to as Johnson's trace elements. *Cladophora* sp. did not grow satisfactorily in a more dilute medium developed by Gerloff, Fitzgerald, and Skoog (1950) in studies on the nutrition of blue-green algae.

Several unsuccessful attempts were made to grow Lake Michigan *Cladophora* sp. in Zuraw's-C Medium (1969) reported satisfactory for the culture of *Cladophora glomerata*. Lake Michigan *Cladophora* sp. also failed to make significant growth in Zuraw's medium with 2 ppm vitamin B_1 added and with the iron (Fe) source increased to 1.12 ppm as FeEDTA. Growth did increase somewhat when lake water was added to Zuraw's solution and increased further when the sodium chloride specified was omitted.

INITIAL CULTURE MEDIUM

Cladophora sp. grew reasonably well in a solution containing the concentrations of the major essential elements specified in Gorham's Medium fortified with effluent (10 to 25% by volume) from the Madison, Wisconsin, Sewage Disposal Plant. It also was established that *Cladophora* sp. growth was improved considerably by the addition of 2 ppm vitamin B_1. This combination became the basic medium in initial efforts to isolate and study Great Lakes *Cladophora* sp.

The stimulation of *Cladophora* growth resulting from additions of 25% sewage effluent to the culture medium in three different experiments is shown in Table 1. The sewage effluent increased yields by 300-400%. In further experiments, it was established that additions of water from several local lakes (25-50%) had the same stimulatory effect as sewage effluent (Tables 1 and 2). Lake water rather than sewage effluent was added to the culture medium in most subsequent experiments.

ISOLATION OF UNIALGAL CULTURES

For detailed nutritional studies, it was essential to work with cultures in which only a single species of *Cladophora* was utilizing the nutrients provided. To meet this requirement, *Cladophora* sp. collected from six lakes were isolated in unialgal condition by the following general procedure.

Small (approximately 1 mg dry weight) clumps of *Cladophora* which had been well rinsed in lake water at the sampling site were placed in 25 to 200 ml of sterile culture medium, usually Gorham's Medium diluted with 25% secondary sewage effluent. The algae clumps were thoroughly rinsed in the culture medium and several subcultures were transferred to fresh medium. As soon as long filaments of *Cladophora* developed from the original clump, they were isolated, washed in sterile medium, and subcultured. The resulting growth was periodically checked for algal contamination. All potentially unialgal cultures were transferred to streaked or poured plates of agar medium (1% agar in Gorham's Medium plus 25% sewage effluent). Growths on the agar plates were checked for algal contaminants. All unialgal cultures were transferred to sterile medium for maintenance as stock cultures. Approximately 150 cultures were initially inoculated in isolating unialgal *Cladophora* sp. from each of six lakes, Mendota (Madison, Wis.), Superior, Michigan, Huron, Erie, and Ontario.

ISOLATION OF BACTERIA-FREE *Cladophora*

It was necessary to separate the *Cladophora* sp. from the contaminating bacteria to demonstrate possible roles of bacteria in the algae's growth, for example, in producing stimulatory organic growth factors. Previous work (Fitzgerald and Faust, 1967; Fitzgerald, 1969) showed that selective use of some chemicals, such as quaternary ammonium

Table 1. STIMULATORY EFFECT OF SEWAGE EFFLUENT AND LAKE WATER ON THE GROWTH OF Cladophora sp. IN NUTRIENT CULTURES

Experiment	Supplement to basal medium[a]	Ave. algae dry-wt yield,[b] mg/l
I	None	74
I	25% Sewage effluent	276
II	None	125
II	25% Sewage effluent	352
II	25% Lake Wingra water	228
III	None	62
III	25% Sewage effluent	215
III	100% Lake Mendota water	344

[a] The nutrient medium contained Gorham's Medium major elements plus vitamin B_1 (2 mg/l).

[b] Average of triplicate cultures in each treatment.

Table 2. QUANTITATIVE RESPONSE OF Cladophora sp. TO THE ADDITION OF LAKE MENDOTA WATER TO THE CULTURE MEDIUM

Lake water,[a] %	pH at inoculation	pH at harvest	Ave. algae dry-wt yield,[b] mg/l
0	8.9	8.2	83
5	9.0	8.3	77
10	8.9	8.5	114
25	9.0	8.8	157
50	8.7	9.2	295
90	8.9	9.6	283

[a] Lake water collected Jan. 8, 1974; nutrient medium as specified in Table 1.

[b] Average of triplicate cultures in each treatment.

compounds or chlorine, would decrease the number of bacteria in algal cultures. Preliminary experiments with Lake Michigan *Cladophora* sp. indicated that free available chlorine killed bacteria associated with the algae without destroying the *Cladophora*. Isolates of *Cladophora* sp. from each of the five Great Lakes were treated with chlorine in an effort to free them of bacteria. The procedure employed was to wash a small amount (approximately 10 mg dry weight) of *Cladophora* in two changes of sterile culture medium and then to mix the algae in a sterile Waring Blender, for approximately one minute. This usually was sufficient to produce a suspension of sections of *Cladophora* filaments. Aliquots of the blended *Cladophora* were transferred by a sterile inoculating loop to culture media containing 2.5, 5, or 10 mg of chlorine/1. At each of ten different treatment times, varying from 5 to 120 minutes, a loopful of *Cladophora* filaments was transferred to sterile medium for incubation. After one to four weeks of incubation, those cultures that showed *Cladophora* growth were tested for the presence of bacteria by plating a portion of the *Cladophora* on tryptone, glucose, extract agar (TGE agar). The remaining algae were transferred to sterile culture medium. *Cladophora* cultures which seemed uncontaminated were again tested in the TGE agar and, finally, those cultures which appeared to be bacteria-free were dragged across TGE agar and cultured in sterile medium. The difference in the technique for isolating bacteria-free *Cladophora* and those previously used for other algae resulted from the failure of *Cladophora* to grow on the organic medium used to test for bacteria. Therefore, portions of the *Cladophora* cultures had to be sacrificed in testing for bacterial contaminants.

The general technique described resulted in the isolation of multiple bacteria-free *Cladophora* sp. cultures from Lakes Superior, Michigan, and Erie. Samples from Lakes Huron and Ontario were initially isolated in bacteria-free condition, but after several transfers were found to be contaminated with bacteria again and were discarded. The bacteria-free isolates from Lakes Superior, Michigan, and Erie survived at least three subcultures over a four-month period, and were in a healthy condition in the apparent absence of bacteria.

Some time later, to further check for contamination, portions of the Lake Michigan isolates were transferred to small amounts of a sterile medium containing 10 g trytone, 10 g dextrous anhydrous and 5 g yeast extract added to one liter *Cladophora* Medium I (see Section V). This procedure indicated that all of the Lake Michigan isolates had become contaminated. Maintenance of bacteria-free algae cultures in inorganic media is difficult. Efforts now are underway to re-establish bacteria-free *Cladophora* sp. using the procedure described above and also treatments with mixtures of antibiotics.

SPECIES IDENTIFICATION

Attempts have been made to establish the species of *Cladophora* isolated from Lake Michigan and used in most of the nutrition experiments. According to Whitton (1970), studies on *Cladophora* in the Great Lakes have been mostly with *C. glomerata* and *C. fracta*. Unfortunately, the only available key for *Cladophora* is for European species (Van Den Hoek, 1963). This key leaves little doubt that the *Cladophora* sp. is either *C. glomerata* or *C. fracta* which are rather difficult to distinguish. The diameter of the apical cells is one distinguishing feature. *Cladophora fracta* apical cells usually are 19-24 μ in diameter; *C. glomerata* are 30-40 μ. Measurements on apical cells from a young culture averaged 32.4 μ; the average from an old culture was 36.7 μ. All of the additional morphological and ecological characteristics were consistent with identification as *C. glomerata*, except the formation of zoospores which occurs in both *C. glomerata* and *C. fracta*. Germlings which had the size and appearance to indicate origins from zoospores, swollen apical cells containing spore-like bodies, and structures among the filaments which resemble zoospores have been observed. Actual zoospores have not been detected. The species used in most of these studies was considered to be *Cladophora glomerata*.

MORPHOLOGY

Cladophora exhibits several morphological variations obvious to the eye and under the microscope. In laboratory cultures, the most common forms were:

a. Loosely tangled masses of filaments which can be highly or slightly branched and which vary considerably in size, depending on their age.

b. "Knots" consisting of large filaments tangled and intertwined; usually akinetes with thick walls can be observed.

c. Germlings which are attached to the walls of culture flasks; they usually are unbranched and range from small, spore-like cells to filaments many cells in length.

Preliminary experiments have been carried out to determine if the rate of *C. glomerata* culture varies with the morphological form of the inoculum, if the morphological form of the inoculum persists through a subsequent transfer, and if agitation of cultures during an incubation period influences the morphology of the resulting growth. Results of the limited experiments were not sufficiently conclusive to justify presentation and discussion of the data.

SECTION V

LABORATORY CULTURE OF GREAT LAKES *Cladophora glomerata* IN A DEFINED NUTRIENT MEDIUM

Following isolation and identification of *Cladophora glomerata* from Lake Michigan and its maintenance in unialgal culture in a non-defined medium, studies could be initiated to define the specific nutritional requirements of the alga. The first effort was to identify the stimulatory factors in sewage effluent and lake water.

Sewage effluent and lake water could be beneficial for several reasons. They could satisfy a *C. glomerata* requirement for an organic growth factor other than vitamin B_1. They also could provide an essential inorganic trace element, either a trace element presently unrecognized or an additional amount of a recognized essential element to satisfy a relatively high requirement. In addition, a favorable balance of major essential elements (for example, a high Ca-Mg environment) could be provided by effluent and lake water. They could affect the pH of the medium, influence the availability of Fe, or provide N or another element in a form preferred by *C. glomerata*.

RESULTS

Major Variations in Inorganic Components

The data in Table 3 show the response of *C. glomerata* to several levels of the essential trace elements in the presence and absence of lake water in the culture medium. Average yields with no lake water in the medium (Treatments A-D) were only 60% of yields with lake water added (Treatments E-H). The addition of trace elements did not increase yields either in the absence or presence of lake water.

Even though there was no response to trace elements, they were added to the medium in most subsequent nutrition experiments at 0.02x the concentration specified by Johnson, et al. (1957) for higher plants. This was to insure that any responses in subsequent experiments on organic growth factors would not be limited by inadequate trace elements. There was no indication that the concentrations of the

Table 3. RESPONSE OF Cladophora glomerata TO SEVERAL CONCENTRATIONS OF THE ESSENTIAL TRACE ELEMENTS

Designation	pH at inoc.	pH at harvest	Lake water, %	Trace element concentration[a]	Ave. algae dry-wt yield, mg/l[b]
A	9.2	8.2	0	-----	165
B	9.1	8.3	0	0.01x	141
C	8.8	8.3	0	0.1x	141
D	8.8	8.4	0	0.2x	132
E	8.8	8.6	25	-----	261
F	8.9	8.6	25	0.01x	263
G	8.8	8.6	25	0.1x	238
H	8.9	8.5	25	0.2x	210

[a] "x" refers to the concentration of the trace elements Mn, Zn, Cu, B, Mo, and Cl in Johnson's Medium.

[b] Average of triplicate cultures in each treatment.

trace elements added were toxic to C. glomerata (Table 3).

The possibility of a very high requirement for one or several trace elements also was considered. Analyses of C. glomerata collected from Lake Michigan and of C. glomerata grown in fortified lake water in the laboratory showed that the alga contained relatively high concentrations of Mn and B. Additions of these elements in relatively high concentrations did not increase yields and did not duplicate the beneficial effects of sewage effluent and lake water.

The pH values of cultures receiving lake water (Treatments E-G, Table 3) were slightly higher at harvest than in cultures without lake water. This effect of lake water also was apparent in the data of Table 2. These minor pH differences probably reflect variations in C. glomerata growth rather than a significant influence of the lake water on the medium. The pH of the nutrient medium increases as a result of algae growth.

Past experience indicated that N and Fe sources in the culture medium could have marked effects on C. glomerata growth. For example, some organisms prefer NH_4-N rather than NO_3-N. Several N concentrations were provided as $NaNO_3$, NH_4NO_3, and urea. Unless sewage effluent or lake water was provided, growth was poor in all cultures. There was no preference for a specific form of N. Because of the relatively high pH of the culture medium (8.0-9.5), maintaining adequate Fe in an available form could be difficult. For the culture of macrophytes under similar conditions (Gerloff, 1973), FeEDDHA, a chelate of Fe with ethylene-diamine di-(o-hydroxyphenylacetate), was found to be a more effective iron source than FeEDTA, a chelate with ethylene-diamine tetraacetic acid. An experiment involving various iron sources and concentrations gave no indications that Fe concentrations higher than in Gorham's Medium were beneficial or that FeEDDHA was a better iron source than FeEDTA either with or without the addition of sewage effluent.

Both sewage effluent and lake water also provided relatively high concentrations of Ca and Mg in the medium. Increased Ca and Mg concentrations in Gorham's medium did not duplicate the beneficial effects of the effluent and lake water.

Organic Growth Factor Requirements

The data in Table 4 show the results of two experiments to establish whether the beneficial effects of lake water and sewage effluent were associated with organic or inorganic factors. Volumes of sewage effluent and lake water equivalent to the volumes added to cultures were evaporated to dryness, the residues were ashed in a muffle furnace at 450°C for 6 hours and then were added to C. glomerata cultures. Destruction of organic constituents by ashing markedly reduced Cladophora glomerata growth in both experiments.

Vitamins, plant hormones, and amino acids seemed the most likely trace organic factors destroyed by ashing sewage effluent and lake water. A requirement for vitamin B_1 was initially established when C. glomerata (Ind. 1484, Indiana Culture collection) was grown for

Table 4. DESTRUCTION BY ASHING OF SEWAGE EFFLUENT AND LAKE WATER FACTORS STIMULATORY TO *Cladophora glomerata* GROWTH

Experiment	Treatment	pH at inoc.	pH at harvest	Ave. algae dry-wt yield,[c] mg/l
I[a]	25% sewage effluent	9.3	---	230
I	As A, except ashed	9.2	---	152
II[b]	No lake water added	8.8	8.3	141
II	25% Lake Mendota water	8.8	8.6	238
II	As C, except ashed	9.2	8.6	150

[a] The nutrient medium contained Gorham's Medium major elements plus vitamin B_1 (2 ppm).

[b] The nutrient medium contained Gorham's Medium major elements, 1/2 Johnson's trace elements, and vitamin B_1 (2 ppm).

[c] Average of triplicate cultures in each treatment.

15 days in a solution containing Gorham's Medium major elements and different proportions of secondary sewage effluent with and without vitamin B_1. The results are shown in Figure 1.

It is evident that the cultures to which 2 mg/l of vitamin B_1 were added produced greater yields than those lacking vitamin B_1. Furthermore, adding vitamin B_1, after 9 days, to a culture containing sewage effluent stimulated growth when measured after an additional six days.

The positive response to vitamin B_1 suggested the need to determine whether *C. glomerata* required additional vitamins. After preliminary experiments showed a response to vitamin B_{12}, the experiment reported in Table 5 was carried out. The nutrient media contained 25% lake water. Because of possible destruction during autoclaving at the alkaline pH of the medium, all vitamins were individually sterilized by millipore filtration and added to the autoclaved medium. The results clearly show a requirement of *C. glomerata* for both vitamin B_1 and B_{12}.

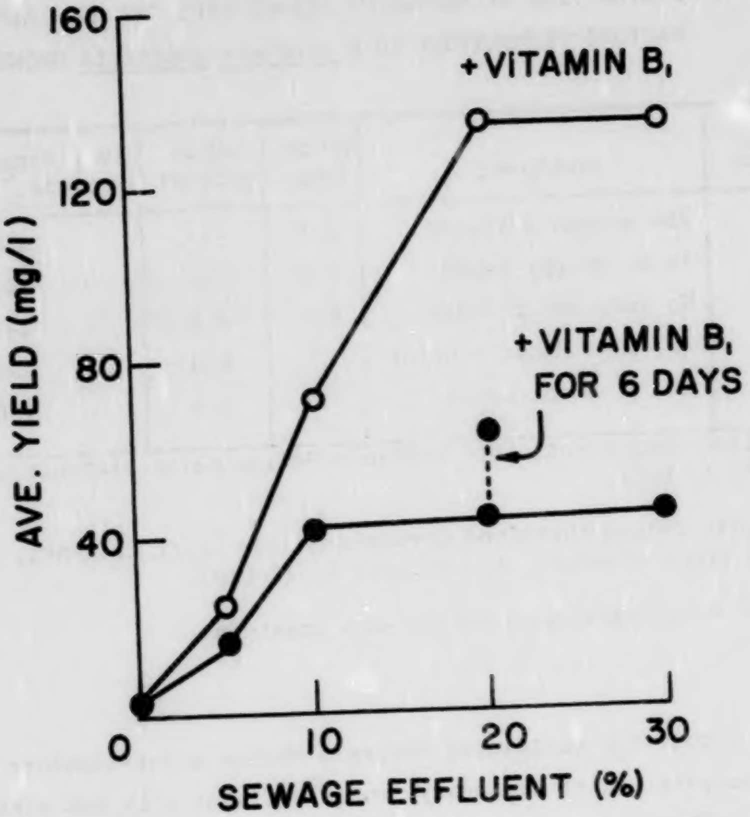

Figure 1. Effect of vitamin B_1 on the growth of Cladophora glomerata (Ind. 1484) in different dilutions of Gorham's Medium major elements and sewage effluent; 15-day culture period.

With only vitamin B_1 or B_{12} added, yields were 53 and 58 mg/l respectively; with both vitamins added, yields increased to 229 mg/l. Additional vitamins did not result in significant yield increases.

The quantitative requirements of Cladophora glomerata isolated from Lake Michigan for vitamins B_1 and B_{12} were established in the experiments reported in Tables 6 and 7, respectively. There was a positive response to each concentration of vitamin B_1 tested, including the maximum concentration of 2.0 mg/l. Maximum yield was obtained with

Table 5. RESPONSE OF Cladophora glomerata TO VARIOUS VITAMINS AND ORGANIC GROWTH FACTORS WHEN GROWN IN CULTURE MEDIUM

Designation	Organic growth factor additions[a]	Ave. algae dry-wt yield,[b] mg/l
A	None	38 a
B	B_1	53 a
C	B_{12}	58 a
D	B_1, B_{12}	229 b
E	B_1, B_{12}, inositol	256 b
F	B_1, B_{12}, nicotinic acid	260 b
G	B_1, B_{12}, p-aminobenzoic acid	263 b
H	B_1, B_{12}, pyridoxine-HCl	244 b
I	B_1, B_{12}, thymine	290 b
J	B_1, B_{12}, folic acid	258 b
K	B_1, B_{12}, DL-Ca-pantothenate	225 b
L	All above vitamins	287 b

[a] The nutrient medium contained Gorham's Medium major elements, 1/50 Johnson's trace elements and 25% lake water.

[b] Average of triplicate cultures in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 6. QUANTITATIVE RESPONSE OF *Cladophora glomerata* TO VARIOUS CONCENTRATIONS OF VITAMIN B_1 IN THE CULTURE MEDIUM[a]

Conc. of vitamin B_1, mg/l	Ave. algae dry-wt yield,[b] mg/l
0	36
0.01	58
0.05	96
0.25	136
1.0	168
2.0	258

[a] The nutrient medium contained Gorham's Medium major elements plus vitamin B_{12} (20 μg/l); 28 day culture period.

[b] Average of triplicate cultures in each treatment.

0.50 μg/l and higher concentrations of vitamin B_{12} in Experiment I of Table 7 and with 0.25 μg/l and higher concentrations in Experiment II. The concentrations of vitamin B_1 and B_{12} to add to *C. glomerata* culture media were established as 2.0 mg/l and 0.5 μg/l, respectively.

An experiment also was carried out to determine if the *Cladophora* sp. isolated from each of the Great Lakes required vitamin B_1. Table 8 summarizes the results. There was a limited amount of growth in the Gorham's major element medium plus 10% lake water, but the addition of vitamin B_1 doubled or nearly doubled the yield of all five *Cladophora* isolates. The requirement of *Cladophora* sp. for vitamin B_1 seems to be general.

To determine if all eight *Cladophora* isolates cultured on this project required vitamin B_{12}, small quantities of each source of *Cladophora* were initially grown in Gorham's major element medium plus vitamin B_1 and then approximately 0.1 mg samples were transferred to cultures with and without 20 μg/l of vitamin B_{12}. After a 32-day growing period the cultures were harvested and the average yields compared (Table 9).

Table 7. QUANTITATIVE RESPONSE OF *Cladophora glomerata* TO VITAMIN B_{12} (CYANOCOBALAMIN) IN THE CULTURE MEDIUM

Experiment	Concentration of vitamin B_{12}, μg/l	Ave. algae dry-wt yield,[c] mg/l
I[a]	0	59
	0.05	121
	0.10	182
	0.50	224
	1.0	203
II[b]	0	151
	0.13	230
	0.25	258
	0.50	257
	1.00	211
	2.00	226
	4.00	246
	8.00	194

[a] The nutrient medium contained Gorham's Medium major elements plus vitamin B_1 (2 mg/l); 22-day culture period.

[b] The nutrient medium contained Gorham's Medium major elements, 1/50 Johnson's trace elements, vitamin B_1 (2 mg/l) and 25% lake water; 21-day culture period.

[c] Average of triplicate cultures in each treatment.

Table 8. THE VITAMIN B_1 REQUIREMENT OF GREAT LAKES Cladophora sp.[a]

Cladophora source	Ave. algae dry-wt yield,[b] mg/l	
	Without vit. B_1	With 2 mg/l vit. B_1
Lake Michigan	74	150
Lake Superior	47	100
Lake Huron	73	123
Lake Erie	41	96
Lake Ontario	14	136

[a] The nutrient medium contained Gorham's Medium major elements, vitamin B_{12} (20 μg/l) and 10% Lake Mendota (Madison, Wis.) water; 14-day culture period.

[b] Average of triplicate cultures in each treatment.

Table 9. THE VITAMIN B_{12} REQUIREMENT OF Cladophora sp.[a]

Cladophora isolate[b]	Ave. algae dry-wt yield,[c] mg/l	
	Without vit. B_{12}	With 20 μg/l vit. B_{12}
Cladophora glomerata (Ind. 1484)	28	100
Cladophora glomerata (Ind. 1488)	8	350
Lake Mendota	5	210
Lake Superior	79	310
Lake Michigan	34	270
Lake Huron	56	150
Lake Erie	19	120
Lake Ontario	100	360

[a] The nutrient medium contained Gorham's Medium major elements plus vitamin B_1 (2 mg/l); 32-day culture period.

[b] Inoculum for this experiment obtained from Cladophora isolates grown in nutrient medium minus vitamin B_{12}.

[c] Average of triplicate cultures in each treatment.

It is evident that all eight isolates of Cladophora sp. responded to and required vitamin B_{12}.

The data in Table 10 show the response of C. glomerata to compounds representing the three major types of plant hormones (auxins, giberellins, and cytokinins). There was no response to the several concentrations of the three compounds tested. Cladophora glomerata apparently is able to synthesize required quantities of these compounds.

DISCUSSION

With the establishment of the vitamin B_1 and B_{12} requirements of Cladophora glomerata, it was possible to produce 200-350 mg oven-dry algae in a liter of defined culture medium after a 21-day culture period. Supplements of lake water and sewage effluent could be omitted.

The demonstrated requirements of Cladophora glomerata for vitamins B_1 and B_{12} are unusual for a freshwater green alga, and probably are the critical factor in the successful laboratory culture of C. glomerata in defined media. At this point in the study, there were no indications that the qualitative or quantitative requirements of C. glomerata for the inorganic components of nutrient media could be considered unusual. It grew satisfactorily at the high pH and with the nutrient concentrations that have been satisfactory for the culture of many other green algae, blue-green algae, and diatoms.

The culture medium for C. glomerata developed in the work described in this section contained the Hughes, Gorham, Zehnder concentrations of the major essential elements (1958), 0.02x the concentrations of the essential trace elements specified by Johnson et al. (1957), 2.0 mg/1 vitamin B_1, and 0.5 μg/1 vitamin B_{12}. Supplemental trace elements also were recommended to maximize the chances for response to other factors. The trace element supplements were derived from two solutions of salts that provided V, Cr, Ni, Co, W, Ti, and Sn (each element at a concentration of 0.001 ppm in the culture solution)

Table 10. RESPONSE OF *Cladophora glomerata* TO ADDITIONS OF PLANT HORMONES TO THE CULTURE MEDIUM[a]

Designation	Hormone addition[b]	Ave. algae dry-wt yield,[c] mg/l
A	None	128
B	None, 25% lake water	221
C	IAA, 10 μM	104
D	GA_3, 1 μM	93
E	BAP, 1 μM	120
F	IAA, 1 μM	136
G	GA_3, 0.1 μM	123
H	BAP, 0.1 μM	128
I	IAA, 1 μM; GA_3, 0.1 μM; BAP, 0.1 μM	127

[a] The nutrient medium contained Gorham's Medium major elements (except 2x Ca), 1/50 Johnson trace elements, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[b] IAA (indole acetic acid); GA_3 (giberellic acid); BAP (benzylaminopurine).

[c] Average of triplicate cultures in each treatment.

and Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se and Be (each at a concentration of 0.0005 ppm) (Arnon, 1938). This culture medium was used extensively in establishing critical element concentrations in *Cladophora glomerata*. It will be referred to as *Cladophora* Medium I.

SECTION VI

CRITICAL CONCENTRATIONS OF ESSENTIAL ELEMENTS FOR *Cladophora glomerata*

Development of a defined culture medium made possible experiments to establish the critical concentrations of various elements for *C. glomerata*. Establishing the critical concentrations was important for several reasons: 1) the nutritional features of *C. glomerata* could be characterized by comparisons of the alga's critical concentrations with critical concentrations for other plants; 2) evaluations of nutrient supplies and potential growth-limiting nutrients by the plant analysis technique would be possible and 3) modifications to improve the culture medium could be suggested because critical concentrations are established by systematic variation of the components of the medium.

EXPERIMENTAL PROCEDURES

The general experimental procedures used in establishing critical concentrations of elements in aquatic organisms have been described by Gerloff and Krombholtz (1966) and Gerloff (1973). To establish the critical concentration of a specific element in *C. glomerata*, the alga was grown at concentrations of that element varying from less than adequate to more than adequate for optimal growth. All other components of the culture medium were held constant at the concentration specified in *Cladophora* Medium I. After a 21-day culture period, the algae were harvested, oven-dried, weighed and then analyzed for the element under study. The critical concentration (the concentration of the element associated with approximately 5% less than maximum yield) was established from a curve obtained by plotting algae yield vs. concentration of the element in the algae.

From experience in the initial studies, the following techniques and procedures were developed for culturing and harvesting *C. glomerata*. Algae were grown in 500 ml flasks containing 200 ml culture medium. In each flask, gentle aeration was provided from a pyrex tube extending through a rubber stopper (Figure 2). Prior to passage through the

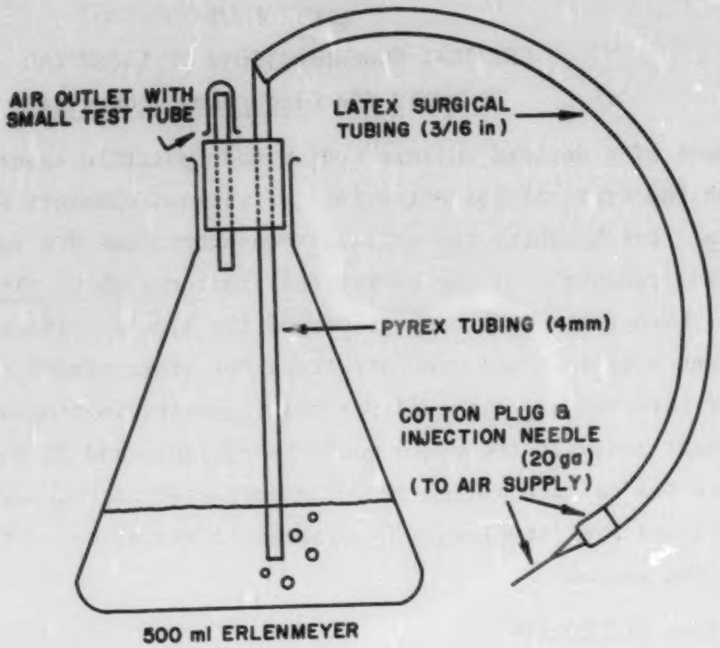
AIR OUTLET WITH SMALL TEST TUBE
LATEX SURGICAL TUBING (3/16 in)
PYREX TUBING (4mm)
COTTON PLUG & INJECTION NEEDLE (20 ga) (TO AIR SUPPLY)
500 ml ERLENMEYER

Figure 2. Apparatus used in the routine laboratory culture of Cladophora sp. in nutritional experiments.

cultures, air was filtered through a polyester fiberfil trap and then through a double-distilled water trap.

Double-distilled water (second distillation from an all-glass still) was used in preparing nutrient media. All glassware was washed, rinsed in distilled water, soaked in approximately 4N HNO_3, and then thoroughly rinsed in distilled water and double-distilled water.

Following preparation, all media and transfer apparatus were autoclaved at 15 psi for 15 minutes.

To inoculate a series of cultures, the main mass of a 3 to 4 week old *C. glomerata* stock culture was transferred with an inoculating loop to a petri dish containing sterile double-distilled water. After rinsing, the filaments were transferred to a second petri dish. Using a sterile forceps and scissors small aliquots (approximately 6-8 mg dry weight) of the alga were removed from the main mass and distributed on a Coors spot-plate. As inoculum, uniform aliquots were selected and randomly distributed to the 500 ml flasks containing medium (one per flask). Sterile technique and instruments were used throughout this procedure. Inoculum used in trace element experiments (Mn, Zn, B, Cu, and Mo) was derived from algae grown in culture medium from which the trace element to be studied had been omitted.

The algae were cultured at 23°C ± 1° under continuous fluorescent light (T-12 cool-white bulbs) of 350-450 foot-candles intensity. Supplemental incandescent light was not provided.

The algae were harvested by filtering cultures through 270 mesh nylon net supported in a Buchner funnel in a suction flask. Considerable effort often was required to loosen algae filaments from the sides of the flasks. After harvest, algae were dried for 48 hours in a forced-draft oven at 63-65°C, cooled in a desiccator, and weighed. Entire harvests from individual flasks, or combined harvests from several flasks, could be used as individual samples in chemical analyses. Grinding of samples was not required.

Element concentrations in *C. glomerata* were determined by quantitative procedures in general use for plant analysis. Total N analyses were by a semi-micro Kjeldahl procedure. Phorphorus was determined by a vanado-molybdate yellow-complex procedure following dry ashing of oven-dried algae at 550°C (Jackson, 1958). Potassium analysis was by emission flame photometry of 1*N* ammonium acetate extracts of tissue samples. Tissues were prepared for Fe analysis by dry ashing for 6 hours at 660°C. Iron then was determined as a complex with o-phenanthroline.

Following dry ashing and acid solution of the residues, Ca, Mg, Zn, and Mn were determined by atomic absorption using a Jarrell-Ash instrument. Boron was measured as a curcumin complex (Johnson and Ulrich, 1959) and Mo as a complex with thiocyanate following stannous reduction (Johnson and Arkeley, 1954). Both analyses were on dry-ashed tissues. Analyses for S were by turbidemetric measurements of $BaSO_4$ precipitated in HNO_3-$HClO_4$ digests of plant tissues (Blanchar, Rehm, and Caldwell, 1965). Copper analyses and B analyses on some samples were run by the Wisconsin Alumni Research Foundation Laboratories. Copper was determined by atomic absorption and B by a Jarrell-Ash Multichannel Emission Spectrometer.

RESULTS

Critical N and P Concentrations

The data presented in Table 11 and graphed in Figure 3 are from an experiment to establish the critical concentration of P in *Cladophora glomerata*. Nutrient solution P concentrations in 8 treatments varied from 0.049 to 7.0 ppm. Yields at these P concentrations ranged from 148 to 361 mg dry-weight/l; the P concentrations in the algae varied from 0.04 to 0.54%. Below approximately 0.06% P, *C. glomerata* yield was less than maximum; above 0.06%, yield was at a constant maximum, even though the P concentration in the algae continued to increase to 0.54%. The highest P concentrations were somewhat inhibitory. From the data presented, the critical P concentration was established at 0.06%. This value was verified in a second experiment.

The data presented in Table 12 and plotted in Figure 4 are from an experiment to establish the critical N concentration for *C. glomerata*. The N concentration in *C. glomerata* varied from 0.83 to 4.89%. Below approximately 1.1%, yield was less than maximum and was directly related to the amount of N provided in the culture medium. The critical N concentration was established as 1.1%, a value verified in a second experiment.

Critical Concentrations of Other Elements

Critical concentrations for most of the other essential elements were

Table 11. YIELD AND TOTAL P CONCENTRATION OF *Cladophora glomerata* GROWN IN CULTURE MEDIUM DIFFERING IN P CONCENTRATION

P added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l	Ave. P conc. in algae, %
0.049	148 a	0.04
0.07	174 ab	0.03
0.10	226 b	0.04
0.14	315 c	0.04
0.28	361 c	0.06
0.56	336 c	0.14
1.40	351 c	0.32
7.00	305 c	0.54

[a] The nutrient medium contained Gorham's Medium major elements (-P), 1/50 Johnson's trace elements, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[b] Average of six replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

determined in the experiments summarized in Tables 13-22. The critical concentrations established from all the experiments and the range in concentration of each element in the *C. glomerata* are summarized in Table 23.

The critical concentrations for Zn, Cu, and Mo are reported in Table 23 as equal to or less than specific concentrations. These concentrations were obtained by analyzing *C. glomerata* grown in basal medium to which the trace element under study was not added. Yields were not reduced by omission of these three elements, so the values presented may not be quite the minimum which will result in maximum yield. However, to obtain more specific critical concentrations would have involved considerable effort and time in purifying culture media of Cu, Zn, and Mo to the point that deficiency could be demonstrated.

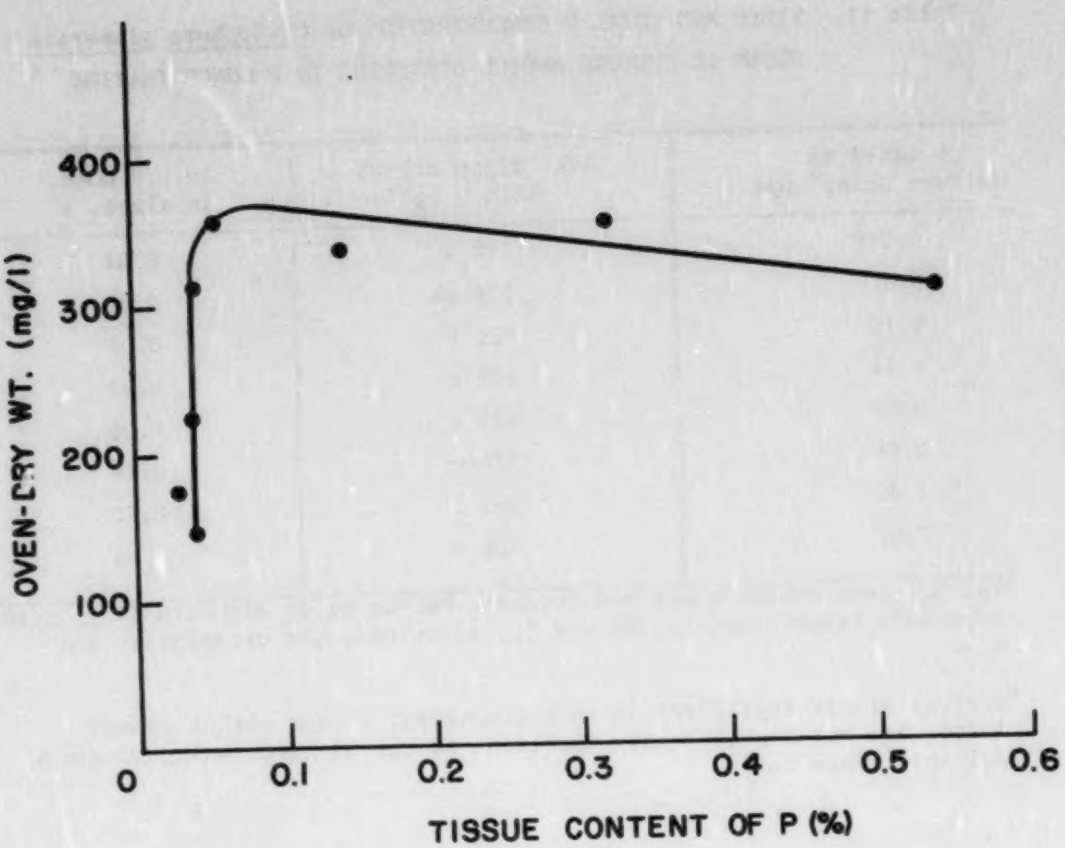

Figure 3. The relationship between oven-dry yield and P concentration in Cladophora glomerata after culturing in solutions varying in P concentration.

Because a considerable part of the project would have been required for just these experiments, the above procedure seemed justified. It also seemed justified to conclude that supplies of certain elements were unlikely to limit growth if the concentrations of those elements in Cladophora sp. samples from the Great Lakes considerably exceeded the concentrations in the algae when grown in unpurified culture media

Table 12. YIELD AND TOTAL N CONCENTRATION OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN NO_3-N CONCENTRATION

N added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l	Ave. N conc. in algae, %
1.0	74 a	0.93
1.6	183 bc	0.83
2.5	222 cd	0.96
3.3	225 cd	1.32
4.1	242 d	1.52
5.6	230 cd	2.35
8.2	176 bc	3.87
10.7	184 bc	4.30
21.0	162 b	4.89
82.0	158 b	4.51

[a] The nutrient medium contained Gorham's Medium major elements (-N), 1/50 Johnson's trace elements, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[b] Average of four replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

which supported maximum growth.

DISCUSSION

As will be discussed in Section X, the critical concentrations established for Cladophora glomerata were used in evaluating nutrient supplies and growth-limiting nutrients in the Great Lakes. In addition, comparisons of these critical concentrations with those established for other species, particularly other algae and aquatic organisms, permit evaluations of the inorganic nutrient requirements of C. glomerata.

The 1.1% critical N and the 0.06% critical P concentrations, and consequently the total requirements for these elements, in C. glomerata are relatively low. For comparison, the critical N concentration in

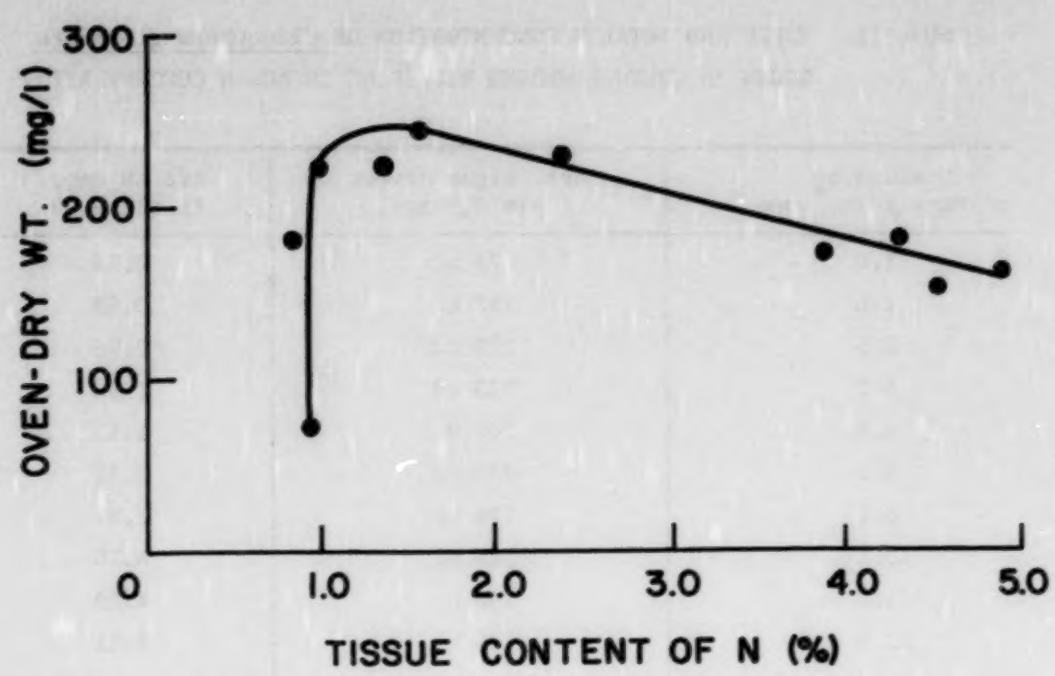

Figure 4. The relationship between oven-dry yield and total N content of Cladophora glomerata after culturing in solutions varying in NO_3-N concentration.

Draparnaldia plumosa, another filamentous green alga, was 2.30% and the critical P concentration was 0.18% (Gerloff, 1975). Critical N and P concentrations in the bloom-producing blue-green alga Microcystis aeruginosa were 4.00% and 0.12%, respectively (Gerloff and Skoog, 1954).

In some macrophytes, the critical N and P concentrations exceeded the C. glomerata values. However, those for Myriophyllum spicatum (0.75% N

Table 13. YIELD AND K CONCENTRATION OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN K CONCENTRATION

K added to culture soln,[a] ppm	Ave. algae dry-wt yield, mg/l	Ave. K conc. in algae, %
0.045	43 b	1.09
0.09	51 b	0.99
0.18	51 b	1.10
0.90	104 b	1.22
1.8	160 c	1.30
2.7	185 c	1.70
3.6	187 c	2.12
5.4	197 c	2.84
9.0	246 c	3.23
18.0	195 c	4.74

[a] The nutrient medium contained Gorham's Medium major elements (except -K and 8.2 ppm N), 1/50 Johnson's trace elements, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[b] Average of ten replicates in each treatment (needed to obtain adequate material for K analysis).

[c] Average of four replicates in each treatment.

and 0.07% P) were comparable to the C. glomerata critical concentrations (Gerloff, 1975).

Algae characteristically have relatively low Ca requirements as shown by variations in critical concentrations from 0.00 to 0.06% in six species studied by Gerloff and Fishbeck (1969). In comparison, the 0.30% for C. glomerata seems relatively high. The 0.15% Mg critical concentration is comparable to the average value of 0.20% for the six algae species in the same study. The critical K concentration in the six algae varied considerably, from 0.25 to 2.40%. The 1.30% value for C. glomerata approximates the average of 1.04% for the six species.

Table 14. YIELD AND Ca CONCENTRATION OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN Ca CONCENTRATION

Ca added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l	Ave. Ca conc. in algae, %
0.49	11 a	0.20
0.97	108 b	0.25
2.4	130 b	0.30
4.9	227 c	0.35
7.3	270 c	0.39
9.7	236 c	0.56
19.4	237 c	0.83
29.1	240 c	1.06

[a] The nutrient medium contained Gorham's Medium major elements (-Ca), 1/50 Johnson's trace elements, B_7 and C_{13} solutions and vitamins B_1 and B_{12}.

[b] Average of four replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

Several C. glomerata critical concentrations were high, relatively, and can be considered very unusual. This is true of the 1.50% value for S. Sulfur critical concentrations and requirements in plants usually are comparable to or somewhat below P requirements. The critical S concentration in Elodea occidentalis, the only aquatic plant for which information is available, was 0.08% (Gerloff, 1975). Sulfur concentrations in deficient agricultural and horticultural crops generally were less than 0.20% (Chapman, 1966).

The 110 ppm B critical concentration in C. glomerata is tremendously high in comparison with critical concentrations in other organisms. Gerloff (1968) in studies on the B nutrition of algae found that B was neither taken up nor required by the three species of green algae tested, both unicellular and filamentous forms; in contrast, B was an

Table 15. YIELD AND Mg CONCENTRATION OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN Mg CONCENTRATION

Mg added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l	Ave. Mg conc. in algae, %
0.08	187 a	0.05
0.15	266 ab	0.08
0.30	328 bcd	0.14
0.60	319 bc	0.18
0.75	392 bcd	0.15
1.50	429 cd	0.26
7.50	454 d	0.37

[a] The nutrient medium contained Gorham's Medium major elements (except -Mg, 16.4 ppm N and 2.8 ppm P), 1/5 Johnson's trace elements, B_7 and C_{13} solutions and vitamins B_1 and B_{12}.

[b] Average of six replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

essential element for three species of blue-green algae and the element was accumulated by cells to relatively high concentrations. Critical B concentrations for agricultural and horticultural plants vary from approximately 4.0 to 30.0 ppm (Chapman, 1966); dicotyledonous plants in general have higher requirements than monocotyledons. The very high B requirement of C. glomerata not only contrasts with the lack of its essentiality for other green algae that have been tested but seems to be considerably higher than reported for any other plant species.

To check on the very high B critical concentration established by analysis of the algae with a colorimetric procedure, additional B deficient C. glomerata were grown and submitted to the Wisconsin Alumni Research Foundation Laboratories for analysis with a Jarrell-Ash Multichannel Emission Spectrometer. The values were lower than

Table 16. YIELD AND S CONCENTRATION IN *Cladophora glomerata* GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

S added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l		Ave. S conc. in algae, %	
	Exp. I	Exp. II	Exp. I	Exp. II
0.97	59 a	----	1.84	----
1.21	----	93 a	----	1.58
1.90	146 b	----	1.42	----
2.43	----	192 b	----	1.27
2.90	244 c	----	1.22	----
4.85	----	316 c	----	1.40
9.70	436 d	429 d	1.77	1.67
14.55	----	330 cd	----	2.30
19.40	----	393 cd	----	2.32

[a] The nutrient medium contained Gorham's Medium major elements (-S), 1/5 Johnson's trace elements, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[b] Average of six replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

in the reported experiment but still far above concentrations reported in B-deficient plants of other species.

Cladophora glomerata requirements for Fe and the trace elements Mn, Cu, Zn, and Mo were quite comparable to those for the macrophyte *Elodea occidentalis* (Gerloff, 1975) and for economic plants (Chapman, 1966). However, the 15 ppm critical Mn concentration is approximately 4x the value established in *Elodea occidentalis*.

Nitrogen and phosphorus usually are given primary consideration in evaluating possible growth-limiting nutrients in lakes and streams. Knowledge of the critical concentrations makes this possible by the plant analysis technique. The very high and unusual critical concentrations for S and B suggest that supplies of these elements in

Table 17. YIELD AND Fe CONCENTRATION OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN Fe CONCENTRATION

Fe added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l	Ave. Fe conc. in algae, ppm
0.00224	140 a	55
0.0056	193 ab	37
0.0112	245 b	36
0.056	351 c	93
1.12	371 c	1110

[a] The nutrient medium contained Gorham's Medium major elements (except -Fe, and 8.2 ppm N), 1/50 Johnson's trace elements, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[b] Average of eight replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

relation to C. glomerata requirements also must be carefully considered in evaluating the nutrition of the alga in the Great Lakes.

The systematic variations of nutrients in the critical concentration experiments suggested modifications of the initial culture medium would be desirable. Those modifications which became a part of the general culture technique are indicated as footnotes to the appropriate tables.

Table 18. YIELD AND Mn CONCENTRATION OF *Cladophora glomerata* GROWN IN CULTURE MEDIUM DIFFERING IN Mn CONCENTRATION

Mn added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l	Ave. Mn conc. in algae, ppm
0.00	225	7.8
0.00054	273	7.2
0.0027	264	14.7
0.0054	293	13.7
0.027	479	17.4
0.27	453	180
2.70	129	4690

[a] The nutrient medium contained Gorham's Medium major elements (except 8.2 ppm N), 1/50 Johnson's trace elements (-Mn), B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[b] The data were obtained from two separate experiments. Some of the treatments were not repeated in both experiments. There were eight replicates of each treatment in each experiment.

Table 19. YIELD AND Zn CONCENTRATION OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN Zn CONCENTRATION

Zn added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l	Ave. Zn conc. in algae, ppm
0.00	556 a	5.3
0.0026	563 a	5.6
0.13	517 a	38.2
0.65	245 b	1039

[a] The nutrient medium contained Gorham's Medium major elements (except 8.2 ppm N), 1/50 Johnson's trace elements (-Zn), B_7 and C_{13} solutions and vitamins B_1 and B_{12}.

[b] Average of twelve replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 20. YIELD AND Cu CONCENTRATION OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN Cu CONCENTRATION

Cu added to culture soln,[a] ppm	Ave. algae dry-wt yield, mg/l	Ave. Cu conc. in algae, ppm
0.00	359 a	8.6
0.00006	358 a	4.9
0.0006	372 a	8.5
0.03	350 a	14.6
0.30	23 b	----
3.00	Insignificant growth	----

[a] The nutrient medium contained Gorham's Medium major elements, 1/50 Johnson's trace elements (-Cu), B_7 and C_{13} solutions and vitamins B_1 and B_{12}.

[b] Average of eight replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 21. YIELD AND Mo CONCENTRATION OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN Mo CONCENTRATION

Mo added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l	Ave. Mo conc. in algae, ppm
0.00	584 a	1.39
0.00002	524 a	0.78
0.0002	584 a	0.90
0.002	546 a	3.6
0.01	619 a	14.0
1.00	538 a	1360

[a] The nutrient medium contained Gorham's Medium major elements (except 8.2 ppm N), 1/50 Johnson's trace elements (-Mo), B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[b] Average of eight replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 22. YIELD AND B CONCENTRATION OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN B CONCENTRATION

B added to culture soln,[a] ppm	Ave. algae dry-wt yield,[b] mg/l	Ave. B conc. in algae, ppm
0.00	127 a	211
0.00054	144 a	178
0.0054	335 b	116
0.054	470 c	110
0.27	492 c	180

[a] The nutrient medium contained Gorham's Medium major elements (except 8.2 ppm N), 1/50 Johnson's trace elements (-B), B_7 and C_{13} solutions, and vitamins B_1 and B_{12}. Culture flasks were low-boron, alkali-resistant glassware.

[b] Average of eight replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 23. CRITICAL CONCENTRATIONS AND THE RANGE OF CONCENTRATIONS OF ESSENTIAL NUTRIENT ELEMENTS IN *Cladophora glomerata*

Element	Critical concentration	Range in concentration
N	1.10%	0.83 - 4.89%
P	0.06%	0.04 - 0.54%
Ca	0.30%	0.20 - 1.06%
Mg	0.15%	0.05 - 0.37%
K	1.30%	0.99 - 4.74%
S	1.50%	1.22 - 2.32%
Fe	45 ppm	36 - 1110 ppm
Zn	$\leq$ 5.0 ppm	5.3 - 1039 ppm
Mn	16 ppm	7.2 - 4690 ppm
Cu	$\leq$ 6.0 ppm	4.9 - 14.6 ppm
Mo	$\leq$ 0.7 ppm	0.7 - 1360 ppm
B	110 ppm	110 - 211 ppm

SECTION VII

RECOMMENDED CULTURE MEDIUM FOR *Cladophora glomerata*

Systematic variation of element levels in the critical concentration experiments indicated the optimum concentration of each essential element for maximum *Cladophora glomerata* growth during the 21-day culture period. The experiments described in this section were concerned primarily with improving the *Cladophora glomerata* culture medium through modifications suggested by the data on optimal concentrations of individual elements. Because of the increased growth in this revised medium, it also was necessary to determine if further modifications of the organic and buffering components would be desirable. For example, vitamin concentrations that had been adequate to produce 250 mg oven-dry algae per liter (Section V) might be inadequate for twice that yield. From these efforts, a recommended culture medium for *Cladophora glomerata* was derived.

The techniques used in growing, harvesting, and analyzing *C. glomerata* were in general those described in Section VI.

RESULTS

Inorganic Constituents

The critical concentration experiments indicated that several elements were present in *Cladophora* Medium I at much higher concentrations than required for the yields obtained. For example, yields actually were decreased by the specified concentrations of N and P. In addition, the unusually high requirements of *C. glomerata* for several elements suggested that the concentrations present in *Cladophora* Medium I might be inadequate. The data in Table 24 show *C. glomerata* response to variations in the inorganic components of the culture medium suggested by the critical concentration experiments.

Lowering the N concentration from 82 ppm to 8.2 ppm and the P from 7.0 to 1.4 ppm in Experiment I did not significantly reduce the yield of *C. glomerata*. This was as anticipated because the 1.1% critical N concentration indicates 8.2 ppm N in the culture medium

Table 24. VARIATIONS IN THE CONCENTRATIONS OF INORGANIC CONSTITUENTS TO IMPROVE THE CULTURE MEDIUM FOR *Cladophora glomerata*

Experiment	Treatment designation	Medium modification	Ave. algae dry-wt yield,[a] mg/l
I	A	*Cladophora* Medium I, 8.2 ppm N, 7.0 ppm P	245 a
	B	As A, but 8.2 ppm N, 1.4 ppm P	234 a
	C	As A, but 16.4 ppm N, 2.8 ppm P	322 a
II	A	*Cladophora* Medium I	353 a
	B	As A, but 10 x trace elements	438 a
	C	As A, but 2x Ca, 2x Mg, 2x K, 2x S	365 a
	D	As A, but 2x Ca, x/2 Mg, x/2 K, x/2 S	223 b
III	A	*Cladophora* Medium I, but 10x trace elements	466 a
	B	As A, but 2x Ca, 2x Mg, 2x K	437 a
	C	As A, but 2x S	528 a
	D	As A, but 2x Ca, 2x Mg, 2x K, 2x S	493 a
IV	A	*Cladophora* Medium II, 9.7 ppm Ca, 16.4 ppm N	338 a
	B	As A, but 23.4 ppm Ca, 16.4 ppm N as $Ca(NO_3)_2$	454 a
	C	As A, but 23.4 ppm Ca as $CaCl_2$	374 a
V	A	*Cladophora* Medium I, Na_2CO_3 & Na_2SiO_3	362 a
	B	As A, minus Na_2CO_3	326 a
	C	As A, minus Na_2SiO_3	324 a
	D	As A, minus Na_2CO_3 & Na_2SiO_3	165 b
	E	As A, but 2x Na_2CO_3, minus Na_2SiO_3	274 ab

[a] Yields within each experiment with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

would provide adequate N for approximately 750 mg C. glomerata oven-dry yield per liter of medium; the 1.4 ppm P would be adequate for 2,300 mg algae at the 0.06% critical concentration. However, the algae were greenish-yellow rather than green at the reduced N and P concentrations. Therefore, the N concentration in the recommended medium was increased to 16.4 ppm; P was increased to 2.8 ppm.

The high requirements of C. glomerata for B and Mn suggested that culture medium concentrations of those elements should be increased. When concentrations of all the trace elements were increased by 10x (Treatment B, Experiment II), average yield was increased from 353 to 438 mg/l. This difference was not statistically significant, probably due to the low number of replicates within treatments. However, in the absence of toxic effects, the recommended concentration of all the essential elements was increased by 10x. The level would insure that responses due to variations in other constituents would not be inhibited by trace element deficiencies.

The effects of varying the concentrations of the major essential elements other than N and P are shown in data from Experiments II and III. Doubling the concentrations of Ca, Mg, K, and S specified in Cladophora Medium I did not significantly increase yields; halving the concentration of Mg, K, and S significantly lowered yields (Treatments C and D, Experiment II). Reference to the S critical concentration experiment suggests the decreased yield probably was due to inadequate S. The results from Experiment III show that with the higher level of trace elements, doubling the concentration of Ca, Mg, K and S did not result in a significant yield increase in any treatment. As a result, Gorham's concentrations of Mg, K, and S are specified in Cladophora Medium II. Based on critical concentrations, these amounts are adequate for considerably greater yields than the 500 mg/l currently obtained but are not inhibitory.

To keep the total salt concentration low, it is desirable to utilize salts in a culture medium which individually provide two essential elements. This is a factor that determines the concentrations of some elements in culture media. For example, because C. glomerata has a relatively high S requirement and $MgSO_4$ is the S source, the amount of Mg provided also is relatively high. The desire to reduce the total salt concentration also accounts for the increase in the Ca concentration from 9.7 ppm in Cladophora Medium I to 23.4 ppm in Medium II. In the Hughes, Gorham, and Zehnder Medium (1958) from which Cladophora Medium I was derived, Ca is obtained from $CaCl_2$ and N from $NaNO_3$. An alternative which is used in many culture media is to derive both Ca and N from $Ca(NO_3)_2$. A test of this modification (Experiment IV, Table 24) indicated yield was not reduced when 16.4 ppm N was provided as $Ca(NO_3)_2$ in Treatment B and Ca, as a result, was increased to 23.4 ppm. Increasing Ca to 23.4 ppm as $CaCl_2$ (Treatment C) also was not detrimental. As a result, $Ca(NO_3)_2$ is the specified source of both Ca and N in the revised medium.

Buffer Components

Sodium carbonate and sodium silicate are added to C. glomerata culture media primarily to provide alkaline buffering. It seemed desirable to confirm the beneficial effects of these salts, because they provide relatively high concentrations of Na which could be toxic to the algae. In addition, in trace element experiments, purification of the culture medium might be less difficult if Na_2CO_3, Na_2SiO_3, or both could be omitted.

The data from Experiment V show that either Na_2CO_3 or Na_2SiO_3 can be omitted from the medium without reduction in yields (Treatments A, B, and C), but that omission of both salts reduced yield to approximately 50% of maximum (Treatment D). Doubling the amount of Na_2CO_3 and omitting Na_2SiO_3 (Treatment E) did not significantly affect yields. These results have been verified in several experiments.

Unrecognized Essential Trace Elements

In studying the culture requirements of any algae, it must be recognized that the organism might require one or even several trace elements currently not recognized as generally essential for plants. For this reason, the effects on C. glomerata of elements which have been shown essential for only specific organisms or which have been suggested but not proven as essential were determined. Mixtures of these elements are commonly designated as A-Z solutions (Hewitt, 1966). Furthermore, the components of A-Z solutions commonly are divided into several subgroups to facilitate identification of any elements which give positive growth responses.

The results of two experiments on the response of C. glomerata to trace element supplements are presented in Table 25. The results from the two experiments are not in complete agreement. The data from Experiment I show that an element in each of the B_7 and the C_{13} solutions, when provided at the proper concentration, significantly increased C. glomerata growth (Treatments A, B, E, and F). Experiment II verified a stimulatory effect of the C_{13} solution but not the B_7 (Treatments A and E).

The results with supplemental trace elements justify routine addition of these solutions to the culture medium until further studies positively establish the response and permit identification of the specific element or elements involved. These studies will require considerable time and effort, because they should involve purification of the culture medium and environment by the techniques used in the most sensitive trace element studies (Hewitt, 1966).

Organic Growth Factors

Because of increases in Cladophora glomerata yields resulting from manipulations of the inorganic components of the medium, it seemed desirable to determine if the specific vitamins and the concentrations of those vitamins established as adequate in early experiments were adequate in the new medium.

As in other experiments, growth was stimulated tremendously by vitamin B_1 (Treatments A and B, Table 26). The additional yield increase resulting from vitamin B_{12} (Treatment C) was not statistically significant. The critical point of this experiment was the lack of a significant response from additional vitamins when autoclaved separately from the medium to minimize breakdown.

Table 25. YIELD OF *Cladophora glomerata* GROWN IN CULTURE MEDIUM CONTAINING SEVERAL CONCENTRATIONS OF POTENTIALLY ESSENTIAL MICRONUTRIENTS

Designation	Treatment	Ave. algae dry-wt yield,[a] mg/l	
		Exp I	Exp II
A	*Cladophora* Medium I	255 a	352 a
B	As A, plus B_7[b] (0.01 ppm)	332 b	321 a
C	As A, plus B_7 (0.002 ppm)	313 a	-----
D	As A, plus C_{13}[c] (0.005 ppm)	311 a	-----
E	As A, plus C_{13} (0.001 ppm)	386 b	445 b
F	As A, plus B_7 (0.01 ppm) & C_{13} (0.005 ppm)	379 b	-----
G	As A, plus B_7 (0.01 ppm) & C_{13} (0.001 ppm)	-----	393 a

[a] Average of ten replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

[b] The B_7 trace element mixture contains the following elements: V Cr, Ni, Co, W, Ti, and Sn.

[c] The C_{13} trace element mixture contains the following elements: Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, and Be.

The lack of a statistically significant response of *C. glomerata* to vitamin B_{12} (Treatments B and C) contrasts with the positive responses to the vitamin in experiments from initial stages of developing a culture medium for the organism (Section V). The difference probably is associated with a failure to obtain inoculum for the experiment reported in Table 26 from a *C. glomerata* culture to which vitamin B_{12} was not added. Considerable B_{12} may have been carried along with the inoculum. A difference in the bacteria, which produce vitamin B_{12}, associated with the algae over a period of time is a less likely

Table 26. RESPONSE OF Cladophora glomerata TO SEVERAL COMBINATIONS OF VITAMINS ADDED TO AN IMPROVED CULTURE MEDIUM

Designation[a]	Vitamin supplement	Ave. algae dry-wt yield,[b] mg/l
A	None	140 a
B	As A, plus vitamin B_1	414 b
C	As A, plus B_1 and B_{12}	529 b
D	As A, B_1 and B_{12} plus additional vitamins[c] autoclaved separately	450 b

[a] Inoculum for this experiment was not cultured in medium lacking in vitamin B_{12}.

[b] Average of five replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

[c] For a listing of the additional vitamins see Table 5.

but possible explanation. The vitamin B_{12} requirement of Lake Michigan C. glomerata can be more positively established when a bacteria-free culture is obtained. General experience on this project indicates that C. glomerata growth is improved by vitamin B_{12}. Until additional information indicates otherwise, the addition of vitamin B_{12} to the culture medium is recommended.

In studies reported in Section V on the quantitative requirement of C. glomerata for vitamin B_1, the alga responded positively to the highest concentration tested (2 mg/l). In an additional test reported in Table 27, the response to vitamin concentration above 2 mg/l was determined. Although yield differences at 2 and 4 mg/l were not statistically significant (Treatments D and E), yields were in general greater in the treatments providing more than 2 mg/l of vitamin B_{12}. The yield at 2 mg/l (Treatment D) was 321 mg of C. glomerata; the average yield

Table 27. RESPONSE OF Cladophora glomerata TO VARIOUS CONCENTRATIONS OF VITAMIN B_1 IN CULTURE MEDIUM II

Designation	Conc. of B_1, mg/l	Ave. algae dry-wt yield,[a] mg/l
A	0.0	110 a
B	0.5	280 ab
C	1.0	295 ab
D	2.0	321 b
E	4.0	442 b
F	6.0	308 ab
G	8.0	465 b
H	10.0	476 b

[a] Average of five replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

at higher B_1 concentrations (Treatments E, F, G, and H) was 423 mg. Because of this response and the absence of adverse effects at concentrations as high as 10 mg/l, the recommended concentration of vitamin B_1 in Cladophora Medium II was increased to 4 mg/l. Requirements may be lower if vitamin B_1 is sterilized separately from the medium. Autoclaving of vitamin B_1 in Medium II is recommended because of convenience.

Unrecognized Growth Factors

In initial efforts at culturing C. glomerata, both lake water and extracts of dried algae provided factors that stimulated C. glomerata growth. As a final aspect of developing an improved culture medium, it seemed desirable to determine if the stimulatory effects of lake water and hot water algae extracts still would be observed when added to the improved medium.

The data in Table 28 show the yield responses from adding several concentrations of a hot-water extract of oven-dried C. glomerata to C. glomerata cultures. Yields increased with increasing concentrations of extract (Treatments A-D), with the 50 ml addition showing statistical significance. The most significant aspect of the results seems to be that when volumes of extract equivalent to the volumes in Treatments B-D were ashed and the residues were added to the culture medium, a significant yield response was retained at the two highest levels of the extract (Treatment F and G). Similar results were obtained when this experiment was repeated.

Table 29 presents the results of an experiment to compare the growth of C. glomerata in Cladophora Medium II and in water from Lake Michigan to which Medium II constituents were added. There was a significantly greater yield when Medium II constituents were added to Lake Michigan water rather than distilled water (Treatments A and B). An unidentified factor in the lake water stimulated the algae. When all inorganic constituents were added to the lake water but vitamin B_1 was omitted, yields were severely reduced (Treatment C) to only 34% of maximum. The reduction in yield to 29% of maximum when only N, P, and Fe and vitamins were added to the Lake Michigan water in Treatment D indicates that one or more inorganic elements other than N, P, or Fe were present in relatively low concentrations in the lake water sample and might limit C. glomerata growth under field conditions.

Treatments E, F, and G in which P, N or Fe were omitted indicate the relative availability of those elements in the sample of Lake Michigan water used. As anticipated, yields were sharply reduced when N, P, or Fe was omitted from the medium. However, surprisingly growth with P omitted (Treatment E) or Fe omitted (Treatment G) was not significantly reduced below Treatment F to which N, P, Fe and vitamins were added but not the other essential elements. This supports the evidence from Treatments B and D that an essential element other than N, P, or Fe limited growth in the water sample. The yield in Treatment F lacking N was significantly less than in any other

Table 28. RESPONSE OF Cladophora glomerata TO ADDITIONS TO THE CULTURE MEDIUM OF HOT WATER EXTRACTS OF OVEN-DRIED C. glomerata

Designation	Treatment	Ave. algae dry-wt yield,[a] mg/l
A	Cladophora Medium II	467 a
B	As A, plus 1 ml algae extract[b]/l	465 ab
C	As A, plus 5 ml extract/l	591 ac
D	As A, plus 50 ml extract/l	614 c
E	As A, plus 1 ml ashed extract/l	494 ab
F	As A, plus 5 ml ashed extract/l	532 bc
G	As A, plus 50 ml ashed extract/l	578 bc

[a] Average of six replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

[b] Algae extract prepared by heating 2.5 g oven-dry C. glomerata in 250 ml distilled water for 60 minutes in Arnold sterilizer.

treatment suggesting N was the primary limiting nutrient in the Lake Michigan sample.

DISCUSSION

The nutrient medium recommended for the culture of Cladophora glomerata as a result of the present studies is presented in Table 30. Cladophora glomerata yields of 500-600 mg dry wt/l are routinely obtained in this medium after a 21-day culture period. Yields in this range are comparable to the growth of other green algae under similar conditions (Gerloff and Fishbeck, 1969) and are approximately double the C. glomerata yields obtained in early phases of this project. With the evolution of Cladophora Medium II, a primary goal of this project was achieved. A defined culture medium had been developed which was suitable for quantitative laboratory experiments with Cladophora glomerata.

Table 29. COMPARISON OF *Cladophora glomerata* YIELDS GROWN IN *Cladophora* MEDIUM II AND IN LAKE WATER FORTIFIED WITH COMPONENTS OF THE MEDIUM

Designation	Treatment	Average algae dry-wt yield,[a] mg/l	Proportion of maximum yield, %
A	*Cladophora* Medium II	420 c	64
B	Lake water[b] + all Medium II constituents	644 d	100
C	As B, except lacking vitamin B_1	216 b	34
D	Lake water + N + P + Fe + vitamins B_1, B_{12}	188 b	29
E	Lake water + N + Fe (-P) + vitamins B_1, B_{12}	245 b	38
F	Lake water + P + Fe (-N) + vitamins B_1, B_{12}	83 a	13
G	Lake water + P + N (-Fe) + vitamins B_1, B_{12}	248 b	39

[a] Average of five replicates in each treatment; yields with a common letter are not significantly different at the 5% level, using Duncan's multiple range test.

[b] Lake water collected 7-3-75 at Green Bay, University of Wisconsin campus.

It probably is incorrect to describe any nutrient solution as an "optimal medium". Unanticipated responses to inorganic and/or organic factors may lead to further yield increases and culture medium modifications. In fact, the results presented suggest that Medium II is not optimal; *Cladophora glomerata* would benefit from further modifications. It seems particularly interesting that several experiments suggested an additional inorganic factor would improve *C. glomerata* yields. This was indicated by the positive response to unidentified trace elements (Table 25), by the beneficial effect

Table 30. COMPOSITION OF RECOMMENDED SYNTHETIC NUTRIENT MEDIUM (MEDIUM II) FOR THE CULTURE OF *Cladophora glomerata*

Salt	Stock solution, g/l	ml stock/l final soln.	Conc. in final solution, ppm
$Ca(NO_3)_2 \cdot 4H_2O$	13.82	10	N - 16.4
Na_2HPO_4	1.28	10	P - 2.8
KCl	3.44	10	K - 18
$MgSO_4 \cdot 7H_2O$	7.50	10	Mg - 7.5
			S - 9.7
$Na_2SiO_3 \cdot 5H_2O$	4.30	10	Ca - 23.4
Na_2CO_3	2.00	10	
Fe citrate-Citric acid[a]	0.60 (of each)	10	Fe - 1.12
EDTA	0.10	10	
KCl[b]	3.728	.2	Cl - 0.35[g]
H_3BO_3[b]	1.546	.2	B - 0.054
$MnSO_4 \cdot H_2O$[b]	0.845	.2	Mn - 0.054
$ZnSO_4 \cdot 7H_2O$[b]	0.575	.2	Zn - 0.026
$CuSO_4 \cdot 5H_2O$[b]	0.125	.2	Cu - 0.006
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$[b]	0.0184	.2	Mo - 0.002
B_7 trace elements[c]	-----	1	(Each) - 0.001
C_{13} trace elements[d]	-----	1	(Each) - 0.0005
Vitamin B_1[e]	-----	10	4
Vitamin B_{12}[f]	-----	5	.0005

[a] Stock solution heated to 80°C, then stored in refrigerator.

[b] 1.2 ml of a mixture of equal volumes of the six stock solutions are added per liter final solution.

[c] The B_7 trace element mixture contains the following elements: V, Cr, Ni, Co, W, Ti, and Sn.

[d] The C_{13} trace element mixture contains the following elements: Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, and Be.

[e] The vitamin B_1 stock solution is prepared fresh, just prior to medium preparation. (40 mg of thiamine•HCl powder per 100 ml d.d. H_2O).

[f] A 0.1 mg/l stock solution of vitamin B_{12} is filtered through a sterile 0.22 μ millipore filter apparatus, stored in the freezer, then thawed and added aseptically to the autoclaved medium.

[g] Cl is also made available in KCl stock solution; the total Cl concentration in the final solution is much greater than provided in the trace element mixture.

of Lake Michigan water (Table 29), the response to ashed extracts of dried C. glomerata (Table 28), and the lack of a response to additional vitamins (Table 26).

Several aspects of the experiment in which components of Cladophora Medium II were added to Lake Michigan water (Table 29) seem of interest in relation to limiting factors for C. glomerata under lake conditions. First, yield was very poor unless vitamin B_1 was included among the supplements added to lake water. In addition, maximum yield was obtained only when inorganic nutrients in addition to N, P, and Fe were made available. This contrasts with the results of comparable experiments with Microcystis aeruginosa, a bloom-producing blue-green alga. Adding only N, P, and Fe to cultures of this organism in lake water resulted in maximum yield (Gerloff and Skoog, 1957). Finally, N rather than P was the primary growth limiting nutrient in the Lake Michigan sample. This was consistent with the results from the comparable test with Microcystis aeruginosa.

As discussed by Gerloff (1969), caution must be exercised in making conclusions on growth-limiting nutrients from experiments on small water samples withdrawn from lakes for laboratory experiments. With this procedure, opportunities for renewal of nutrient supplies in the water are eliminated. Nevertheless, this approach does provide some information on relative supplies of various nutrients in lakes. The data obtained suggest that additional similar experiments with water samples obtained at various sites and at various times of the year would be desirable.

SECTION VIII

Cladophora glomerata RESPONSE TO SEVERAL PHYSICAL ENVIRONMENTAL FACTORS

Non-nutritional factors must be considered in determining optimal conditions for the laboratory culture of an organism. The results of experiments on the response of C. glomerata to variations in temperature, light, and several other physical factors are presented in this section.

Unless indicated, culture techniques and growth conditions were as described in Section VI.

RESULTS AND DISCUSSION

Light and Temperature

Experiments were carried out to determine if Cladophora glomerata showed unusual responses to light or temperature and if the alga was being grown in the laboratory under conditions close to optimal.

Table 31 shows that the temperature (22-23°C) under which C. glomerata has been routinely cultured on the project is optimum or close to optimum. The algae seemed relatively insensitive to high temperature with optimum yields maintained at 30-31°C. This contrasts sharply with the results of Wojick (1972) in which yields of several green algae were severely inhibited at 30°C. Growth of C. glomerata was reduced to only 63% of optimum at temperatures of 16-17.5°C.

The results in Table 32 indicate that C. glomerata is relatively insensitive to variations in light intensity. The different intensities tested were achieved within one growth cabinet by placement of the cultures and by covering some cultures with one or two layers of cheesecloth. Yields at the four light intensities tested (ranging from approximately 200 to 1100 ft. candles) varied from only 363 to 457 mg/l. The differences were small but statistically significant. Maximum yield was under the 630-750 ft. candle treatment which is slightly above the 400-500 ft. candles which had been used for routine culture of C. glomerata. Both the highest (1000-1100 ft. candles)

Table 31. THE GROWTH OF Cladophora glomerata TO THREE RANGES OF TEMPERATURE

Designation	Temperature, °C	Ave. algae dry-wt yield,[a] mg/l
A	22-23	506 b
B	16-17.5	318 a
C	30-31	502 b

[a] Average of six replicates in each treatment; yields with a common letter are not significantly different at the 5% level, using Duncan's multiple range test. The nutrient medium contained Cladophora Medium II elements except 2 ppm vitamin B_1 and 9.7 ppm Ca as $CaCl_2$.

Table 32. THE GROWTH RESPONSE OF Cladophora glomerata TO FOUR LEVELS OF LIGHT INTENSITY

Designation	Light intensity, f.c.	Ave. algae dry-wt yield,[a] mg/l	Color
A	1000-1100	410 c	Yellow
B	630-750	457 d	Yellow-green
C	310-430	384 b	Green
D	160-260	363 a	Dark green

[a] Average of six replicates in each treatment; yields with a common letter are not significantly different at the 5% level, using Duncan's multiple range test. The nutrient medium contained Cladophora Medium II elements except 2 ppm vitamin B_1 and 9.7 ppm Ca as $CaCl_2$.

and the lowest (160-260 foot candles) intensities tested resulted in some yield decreases.

There were marked variations in algae color under the different light intensities. The cultures which were darkest green (Treatments D and C) did not produce the highest yields. Cultures grown at 1000-1100 foot candles were yellow, and yield was also below maximum.

Volume and Aeration of Culture Medium

The primary source of carbon for algae growth in culture media is CO_2 dissolved from the air above the cultures. Most media contain only limited amounts of added bicarbonates or carbonates (15 to 20 mg/l) as a means of buffering the pH to alkaline values. The importance of the CO_2 supplied from the air is demonstrated by the slight growth that develops in rubber-stoppered flasks as compared with the growth in flasks with cotton or plastic-foam closures. The rate at which CO_2 can be replaced in an algal culture depends on the ratio of surface area to total volume of the medium in the flask. As the volume of medium in a culture is increased and the surface area remains the same or is decreased, replacement of utilized CO_2 within the medium is slowed.

Aeration or shaking of cultures does compensate to some extent for the effects of increased solution volumes as shown in Table 33. In this experiment, _C_. _glomerata_ was grown in different volumes of medium which were either aerated or quiet (non-aerated). The growth of _C_. _glomerata_ decreased in both aerated and non-aerated cultures as the volume of the medium increased. When harvested after 14 days of growth, an increase in the amount of medium from 20% of flask volume (100 ml) to 60% (300 ml) resulted in a yield decrease from 170 to 70 mg in the non-aerated cultures and from 245 to 115 mg in the aerated cultures. There was considerably more growth in all of the aerated cultures. When non-aerated cultures from another experiment were harvested after a 24 day period, unreported data, there was only a 35% yield decrease when the medium was increased to 60% of flask volume. These results were similar to those from aerated cultures harvested after a longer culture period. In these studies, the rate of CO_2 supply seems to regulate the rate of _C_. _glomerata_ growth.

In additional experiments to determine the effect of CO_2 supply on rate of _C_. _glomerata_ growth, algae were grown in tubes (38 x 300 mm) containing 150 ml of medium and were aerated either above the medium

Table 33. THE EFFECT OF MEDIUM VOLUME AND AERATION ON THE GROWTH OF *Cladophora glomerata*

Medium volume,[a] ml/500 ml Erlenmeyer	Ave. algae dry-wt yield,[b] mg/l		Effect of volume on yield, % decrease		Effect of aeration on yield, % increase
	Non-aerated	Aerated	Non-aerated	Aerated	
100 .	170	245	0	0	45
200	115	200	32	18	75
300	70	150	59	39	115

[a] The nutrient medium contained Gorham's Medium major elements plus 10% lake water.

[b] Average of triplicate cultures; 14-day culture period.

or by extending a tube to 25 or 60 mm depths in the medium. Air was provided at approximately 200 ml/minute. Increased aeration did not affect the final culture pH. After a 10 day growing period, average yields of 4 cultures in each treatment were 20, 85 and 140 mg/l, respectively, indicating definite increases in rate of *C. glomerata* growth with increased CO_2 supply

Growth Surface

One of the most troublesome aspects of laboratory experimentation with *Cladophora glomerata* has been the poor agreement in yields from replicate cultures within a treatment. As a result, unusually large numbers of replicates have been required to establish statistically significant differences among the treatments of an experiment.

Filaments of *C. glomerata* often grow attached to the sides of pyrex culture flasks. The amount of attached growth has been observed to vary considerably. It was considered possible that algae growth to a degree was determined by the amount of attached growth which in turn was related to differences in the interior surfaces of flasks resulting from use and repeated washing. The algae would more readily attach to rough surfaces.

Table 34. EFFECT OF "ETCHING" ON *Cladophora glomerata* GROWTH

Experiment[a]	"Treatment"	Ave. algae dry-wt yield, mg/l	Range of yield and standard deviation, mg/l
I[b]	1 liter pyrex beaker, normal	206.8	140.8-329.2; 106.0
	1 liter pyrex beaker, etched	320.0	282.6-343.6; 32.7
II[c]	500 ml Erlenmeyer, normal	385.9	200.0-610.5; 155.6
	500 ml Erlenmeyer, etched	572.4	403.5-669.5; 105.9

[a] The nutrient medium contained Cladophora Medium II elements except 2 ppm vitamin B_1 and 9.7 ppm Ca as $CaCl_2$.

[b] Average of three replicates in each treatment.

[c] Average of eight replicates in each treatment.

The data in Table 34 are from two experiments in which *C. glomerata* growth in new glassware and in glassware artificially scratched with emery cloth or a grinding stone was compared. In Experiment I, the algae were grown in 1-liter beakers; in Experiment II, in the usual 500-ml Erlenmeyer flasks. In both experiments, average yields were considerably greater in the etched culture containers. Of more importance, is the decreased variability in yields from the etched flask cultures. In each experiment, both the range in yield and the standard deviation were less in the etched culture vessels. Before specific recommendations can be made, additional similar tests should be carried out.

SECTION IX

CALIBRATION OF FITZGERALD TESTS FOR FIELD EVALUATIONS OF NUTRIENT SUPPLIES

Two types of bioassays have been used on this project to determine whether the growth of Cladophora sp. in specific aquatic environments was limited by supplies of certain nutrients. The plant analysis bioassay was developed for use with aquatic plants by one of the Principal Investigators (Gerloff and Krombholz, 1966; Gerloff, 1969). Application of plant analysis to assaying nutrient supplies for C. glomerata was discussed in Section III. The second bioassay (Fitzgerald tests) which is based on PO_4-P extracted in boiling water and rate of NH_4-N uptake as measures of P and N sufficiency was developed by the other Principal Investigator (Fitzgerald and Nelson, 1966; Fitzgerald, 1969). The laboratory efforts required to calibrate the Fitzgerald tests in evaluating nutrient supplies for Cladophora sp. will be presented in this section.

RESULTS AND DISCUSSION

Extractable PO_4-P

Several experiments were carried out to determine the relationship between algae yield, total P concentration, and extractable PO_4-P when Lake Michigan Cladophora sp. was grown in different solution concentrations of PO_4-P. The results of representative experiments are presented in Figures 5 and 6.

In both experiments, algae yields increased with increasing concentrations of P in the medium up to 0.2-0.3 mg P/1. The total P concentration in the Cladophora sp. which corresponded with slightly less than maximum-yield was approximately 0.10%. This is an additional evaluation of the critical P concentration. It corresponds reasonably well with the 0.06% in earlier experiments of Section VI. The minimum concentration of hot-water extractable PO_4-P associated with maximum yield was approximately 0.07 mg/100 mg algae (%P). This correlates well with results with other algae in which less than 0.08% extractable PO_4-P was considered to indicate the algae were

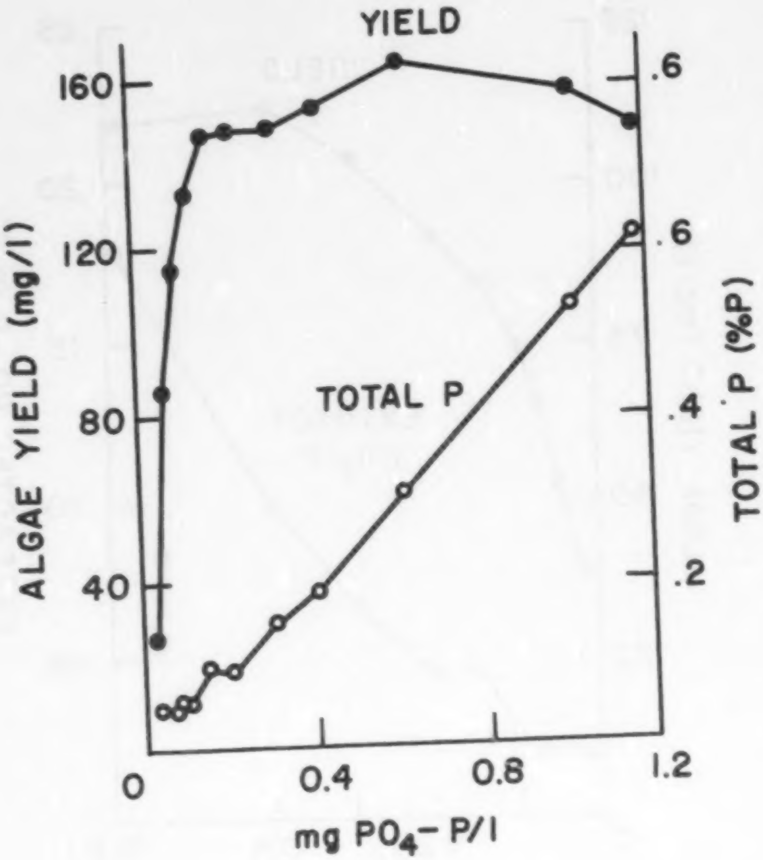

Figure 5. Comparison of yield and total P concentration of Lake Michigan Cladophora sp. after a 17-day culture period in solutions varying in P concentration.

P-limited (Fitzgerald and Nelson, 1966).

To demonstrate that the Cladophora sp. isolated from all five of the Great Lakes had similar P requirements, yields obtained after culture at several PO_4-P concentrations are summarized in Table 35. In general, responses of the five isolates were similar. Comparative yields at 0.05 ppm P probably are of most interest because that approximates a

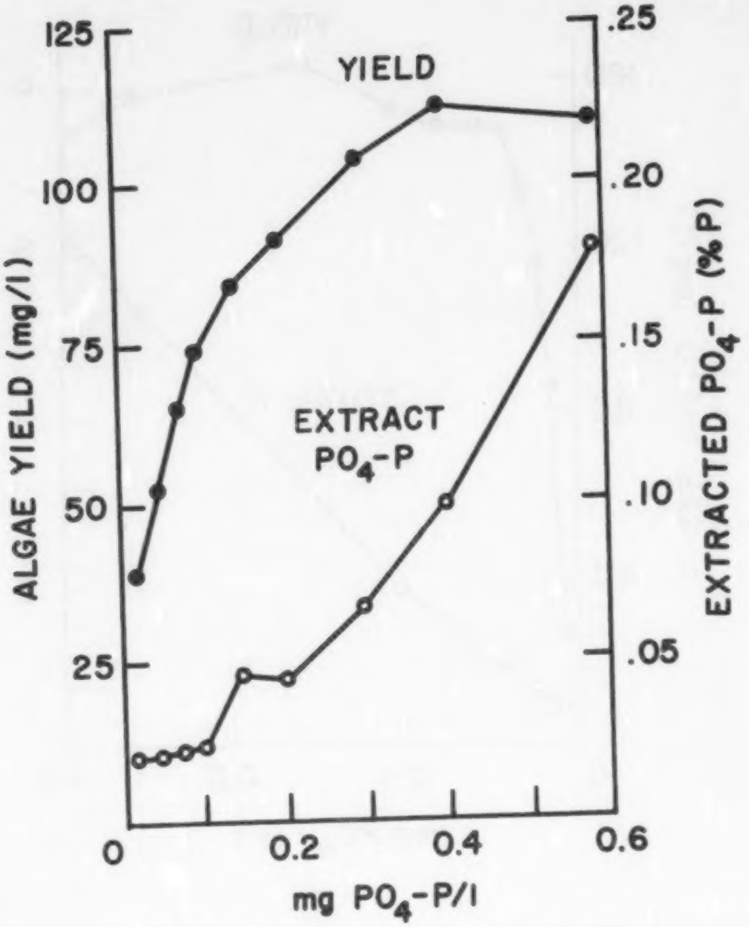

Figure 6. Comparison of yield and extracted PO_4-P of Lake Michigan Cladophora sp. after a 17-day culture period in solutions varying in P concentration.

growth-limiting P supply. Yields of the Lake Huron isolate were the lowest or nearly the lowest at each P concentration.

P Availability from Various Sources

The availability to algae of an essential nutrient in a specific source usually is determined by comparing growth of the algae in

Table 35. THE RESPONSE OF Cladophora sp. ISOLATED FROM EACH OF THE GREAT LAKES TO DIFFERENT P CONCENTRATIONS[a]

Source of Cladophora	Ave. algae dry-wt yield,[b] mg/l		
	0 mg P/l	0.05 mg P/l	0.15 mg P/l
Lake Michigan	18	113	168
Lake Superior	30	97	130
Lake Huron	11	75	115
Lake Erie	18	92	112
Lake Ontario	20	105	142

[a] The nutrient medium contained Gorham's Medium major elements (except 1/2 N and 2x Ca) vitamins B_1 and B_{12} and 10% Lake Mendota water.

[b] Average of triplicate cultures in each treatment; 25-day culture period.

different concentrations of that source and known available sources of the nutrient. The time required for these tests usually exceeds two weeks for Cladophora sp. During this interval non-available sources of a nutrient may break down to usable forms. Therefore, alternative tests that can be carried out rapidly with a minimum requirement for laboratory space are desirable. These relatively simple procedures are not meant to replace growth tests or short-term-exposure-and-growth tests (Fitzgerald, 1970), but can be used for comparative studies and surveys of a large number of potential sources of available nutrients.

It has been demonstrated that Cladophora sp. in which growth is limited by P, N, or Fe are capable of absorbing these nutrients, if they are presented in available forms. Therefore, nutrient-limited field collections of Cladophora sp. were used to evaluate the availability of different nutrient sources.

Approximately 5 mg (dry weight) of P-limited Cladophora sp. from Lake Wingra were added to 500 ml of Gorham's (-P) Medium containing different concentrations of ortho-PO_4, pyro-PO_4, tripoly-PO_4, and phosphite-P. These P sources contained 100, 2.4, 1.6 and 0.4% ortho PO_4-P, respectively. After incubation for 20 hours at 25°C, the amounts of PO_4-P extractable from the algae were determined (Table 36).

The data show that pyro-PO_4 and tripoly-PO_4 are readily available to Lake Wingra *Cladophora* sp.; the P of phosphite is unavailable. These data agree with the results from comparable studies with other algae (Fitzgerald, 1970). The value of the type of information obtained from this test was demonstrated in the failure of algae to utilize some of the P in Yahara River sewer discharge with chemical tests indicated to be soluble PO_4-P (Fitzgerald and Uttormark, 1974).

The availability of different sources of P for the growth of Lake Michigan *Cladophora* sp. also was tested (Table 37). There was an increase in yield with each increase in P concentration as ortho-, pyro-, meta-, and tripoly-PO_4. All of these P sources were available to the *Cladophora* sp. The relatively low amount of growth (43 mg/l) in the presence of 0.4 mg phosphite-P/l was probably due to the ortho-P present (12%). Additional tests demonstrated the low yield was due to P deficiency and not to phosphite toxicity.

An experiment was carried out to test the availability to *Cladophora* sp. of P from the littoral muds of several lakes. Previous tests had indicated that algae could utilize only a small part of the P present in bottom muds (Fitzgerald, 1970). There was no detectable PO_4-P in the water above the sample area in one of the lakes tested (Wingra); P was barely detectable (0.02 mg PO_4-P/l) in the waters of Monona Bay; the overlying waters of Lake Waubesa (east shore) and Lake Kegonsa (east shore) had 0.07 and 0.10 mg PO_4-P/l, respectively. The yields of *Cladophora* sp. from cultures containing lake muds and several concentrations of added P are summarized in Table 38. Comparisons of yields of *Cladophora* sp. from the lake mud cultures with yields from cultures with known concentrations of PO_4-P indicated the concentrations of available P and the percentage of the total P of the lake muds available for algae growth.

Table 36. THE RELATIVE AVAILABILITY OF DIFFERENT SOURCES OF P TO P-LIMITED LAKE WINGRA Cladophora sp.

Sample	Phosphorous source	Phosphorous conc., mg/l	Algal extractable PO_4-P,[a] mg P/100 mg algae
A	None	0.00	0.046
B	Ortho-PO_4	0.04	0.11
C	Ortho-PO_4	0.08	0.16
D	Ortho-PO_4	0.12	0.19
E	Ortho-PO_4	0.16	0.24
F	Pyro-PO_4	0.08	0.18
G	Pyro-PO_4	0.12	0.23
H	Tripoly-PO_4	0.08	0.17
I	Tripoly-PO_4	0.12	0.19
J	Phosphite-PO_4	0.16	0.047
K	Phosphite-PO_4	0.80	0.052
L	None	0.00	0.049

[a] Average of duplicate cultures in each treatment; 5 mg Cladophora sp./ 500 ml Gorham's Medium (-P) after a 20 hr incubation at 25°C.

Table 37. THE AVAILABILITY TO LAKE MICHIGAN Cladophora sp. OF P FROM DIFFERENT SOURCES WHEN MEASURED IN GROWTH TESTS

Source of P[a]	Ave. algae dry-wt yield,[b] mg/l			
	0.05 mg P/l	0.10 mg P/l	0.20 mg P/l	0.40 mg P/l
Ortho-P	18	110	165	--
Pyro-P	--	137	195	--
Meta-P	20	121	177	--
Tripoly-P	42	135	175	--
Phosphite-P	--	---	---	40

[a] The nutrient medium contained Gorham's Medium major elements (except 1/2 N and 2x Ca), vitamins B_1 and B_{12}, and 5% Lake Mendota water.

[b] Yield with no P added was 5 mg/l; average of triplicate cultures in each treatment; 27-day culture period.

Table 38. THE AVAILABILITY OF THE P OF LITTORAL MUDS TO LAKE MICHIGAN Cladophora sp.

Phosphorous source	Conc., mg P/l	Ave. algae dry-wt yield,[a] mg/l	Calculated available P, mg/l	Available P, %
PO_4-P	0	5	----	--
PO_4-P	0.025	26	----	--
PO_4-P	0.050	86	----	--
PO_4-P	0.10	134	----	--
PO_4-P	0.15	150	----	--
Wingra a	0.5	41	0.04	8
Wingra a	1.0	74	0.05	5
Wingra b	0.5	34	0.03	6
Wingra b	1.0	91	0.05	5
Monona Bay a	0.5	37	0.03	6
Monona Bay a	1.0	106	0.06	6
Monona Bay b	0.5	123	0.08	16
Monona Bay b	1.0	133	0.10	10
Waubesa a (W)	0.5	15	0.01	2
Waubesa a	1.0	24	0.02	2
Waubesa b (E)	0.5	139	0.10	20
Waubesa b (E)	1.0	152	0.15	15
Kegonsa a (E)	0.5	241	0.15	30
Kegonsa a	1.0	243	0.15	30
Kegonsa b (SE)	0.5	136	0.10	20
Kegonsa b	1.0	228	0.15	30

[a] The nutrient medium contained Gorham's Medium major elements (-P) and vitamins B_1 and B_{12}; 19-day culture period.

It is evident that very little of the P (less than 10%) was available to Cladophora sp. from Lake Wingra muds, from one Monona Bay sample, and from mud from the west shore of Lake Waubesa. The greater availability of the P (20-30%) in lake muds collected from Lake Kegonsa at a time when the overlying water contained 0.10 mg PO_4-P/1 is not surprising. These muds probably were saturated with P. In contrast, muds from Lake Wingra had already lost any P that could have been exchanged with the overlying water. These results point out that studies of the capacities of lake muds to provide available P for algae growth should be tested at different seasons, so that results when the overlying waters contain PO_4-P can be contrasted with results when the overlying waters have been stripped of available P.

Nitrogen Availability

To utilize rate of NH_4-N uptake as a bioassay of N availability to *Cladophora* sp. it was necessary to establish the specific values that indicated adequate or deficient N supply for *Cladophora* sp. To accomplish this, the algae were grown in different concentrations of NO_3-N in the culture solution, and after a 24-day culture period, yields, total N concentrations, and rates of NH_4-N uptake were determined (Figure 7).

Growth of *Cladophora* sp. increased as the NO_3-N concentration in the medium increased up to approximately 8 ppm. There was a slight further yield increase at higher N concentrations, but not in proportion to the amount of N added. At solution NO_3-N concentrations of less than 8 ppm, total N concentrations in *Cladophora* sp. decreased and NH_4-N absorption rates increased in proportion to the decrease in N in the medium. Therefore, a total N content of 1.8% and an NH_4-N absorption rate in excess of 15 µg N/10 mg algae/hour would define N-limited *Cladophora* sp. The 1.8% critical N concentration is slightly higher than established in experiments in Section VI; the NH_4-N uptake critical value is equal to the value established for other algae (Fitzgerald, 1968).

To evaluate the availability of different sources of N, algae can be grown at various concentrations of the N sources and rates of NH_4-N absorption or algae yields can be compared. Ammonium-N uptake tests were carried out to compare the effectiveness of NH_4-N, nitrite (NO_3-N) glutamate-N, and urea-N as N sources for *Cladophora* sp. Twenty to

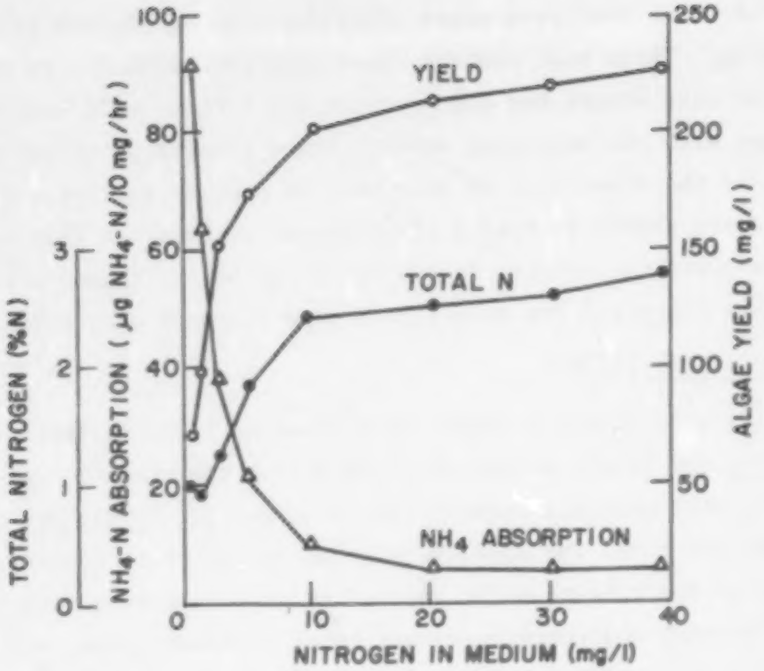

Figure 7. Comparison of yield, total N in the algae, and rate of NH_4-N uptake in the dark when Cladophora sp. was grown at different solution concentrations of NO_3-N.

25 mg samples of Cladophora sp. from Lake Mendota known to be nitrogen limited (47 µg NH_4-N absorbed/10 mg algae/hour) were incubated in 1, 2, and 6 mg N/1 provided from each N source for 22 hours with shaking at 20°C. All three concentrations of NH_4-N and NO_3-N caused reductions in the rates of NH_4-N absorption when tested after the incubation period (1-8 µg NH_4-N/10 mg algae/hour). In contrast, absorption rates with the added glutamate-N were only reduced to 20-32 µg/10 mg/hour

and with the added urea to 34-43 μg/10 mg/hour. Thus, all of the nitrite nitrogen seemed to be available to the nitrogen-limited Cladophora sp., but only part of the glutamate-N and none of the urea-N could be utilized in such short exposure times.

The results of a more definitive test of N availability in which the capacity of C. glomerata to utilize sources of N for actual growth are presented in Table 39. The yield of Lake Michigan Cladophora sp. was obtained with different concentrations of NO_3-N, NO_2-N, NH_4-N, and dried abysmal Lake Waubesa muds (1.3% N).

Yield increased with increasing concentrations of all the sources of N. All four could be used for Cladophora sp. growth. Yield with the highest concentration of NH_4-N, 5 mg N/1, was considerably less than with 2 mg N/1. This is probably due to a lower pH in the media with higher amounts of NH_4-N. The NH_4-N absorbed by algae is replaced with H^+ which results in lowering of the pH. The yield per mg of N was fairly uniform for NO_3^-, NO_2^-, and NH_4-N, but the yield per mg of N from the lake muds was very low, only 1 to 2% of the N of the lake muds being available under the conditions tested. Similar experiments have corroborated the poor availability of the N of lake muds to Cladophora sp. and to other algae under aerobic conditions (Fitzgerald, 1970) and to the aquatic weed Lemna minor (duckweed) (Fitzgerald & Uttormark, 1974).

Iron Availability

The availability to Cladophora sp. of Fe from several sources was measured in tests in which the algae were grown for 24 days at different concentrations of each Fe source. The data obtained (Table 40) show that Fe-EDTA and Fe-citrate gave increasing yields at the concentration of Fe increased, but there was almost no growth when $FeCl_3$ was the Fe source.

In another growth experiment (Table 41), Lake Michigan Cladophora sp. was grown with different concentrations of iron derived from Fe-EDTA or littoral lake muds. By using the yields of the cultures with Fe-EDTA to construct a growth versus concentration of iron relationship,

Table 39. THE AVAILABILITY OF DIFFERENT SOURCES OF NITROGEN TO LAKE MICHIGAN *Cladophora* sp. WHEN MEASURED IN GROWTH EXPERIMENTS

Nitrogen source[a]	Concentration of N, mg/l	Ave. algae dry-wt yield,[b] mg/l
None	0	6
NO_3-N	0.5	62
NO_3-N	1.0	106
NO_3-N	2.0	132
NO_3-N	5.0	131
NO_2-N	0.5	57
NO_2-N	1.0	97
NO_2-N	2.0	111
NO_2-N	5.0	110
NH_4-N	0.5	53
NH_4-N	1.0	87
NH_4-N	2.0	116
NH_4-N	5.0	63
L. Waubesa dried	13.0	25
abysmal muds	26.0	64
(1.3% total N)	52.0	102

[a] The nutrient medium contained Gorham's Medium major elements (-N) and vitamins B_1 and B_{12}.

[b] Average of triplicate cultures; 22-day culture period.

the concentration of available iron in the lake mud cultures could be calculated from algae yields. From these data, the percentage of the iron in the different lake muds which was available for the growth of *Cladophora* sp. also was calculated. Although the *Cladophora* sp. could use the Fe from Fe-EDTA, the iron of the different lake muds tested was no more than 10% available.

Table 40. COMPARISON OF THE EFFECTIVENESS OF SEVERAL SOURCES OF Fe FOR *Cladophora* sp. GROWTH IN NUTRIENT CULTURES

Concentration,[a] mg Fe/l	Ave. algae dry-wt yield,[b] mg/l		
	Fe-EDTA	Fe citrate	$FeCl_3$
0.075	33	13	4
0.15	72	41	3
0.30	87	70	6
0.60	108	100	9

[a] The nutrient medium contained Gorham's Medium major elements and vitamins B_1 and B_{12}.

[b] Average of triplicate cultures in each treatment; 24-day culture period.

Although laboratory tests may indicate that some sources of Fe are not readily available for *Cladophora* sp. growth, these sources may be available in natural environments when certain chelates are added or other algae decay in the immediate environment. However, the relative availability of the iron in different natural environments can be evaluated by analyses of the iron concentrations of *in situ* algae.

Confirmation of Fitzgerald Tests

To confirm the validity of the Fitzgerald bioassays when applied to algae collected from field environments, tests were carried out to demonstrate that algae which are alive but limited by available P, N, or Fe are able to accumulate the nutrients with corresponding measurable enzymatic or nutrient changes when placed in an environment with adequate supplies of N, P, or Fe.

Cladophora sp. from Lake Michigan which were indicated as P-limited by low extractable PO_4-P tests were incubated 14 hours in Lake Michigan water to which different amounts of PO_4-P had been added. The results of extractable PO_4-P analyses of these algae are summarized

Table 41. THE AVAILABILITY OF THE IRON OF LITTORAL MUDS FOR THE GROWTH OF Cladophora sp.

Iron source	Iron added, mg Fe/l	Yield,[a] mg/l	Calculated available Fe, mg/l	Available Fe, %
None	0	5	-----	--
Fe-EDTA	0.075	88	-----	--
Fe-EDTA	0.15	113	-----	--
Fe-EDTA	0.30	138	-----	--
Fe-EDTA	0.45	171	-----	--
Fe-EDTA	0.60	169	-----	--
Wingra a	0.3	26	0.02	7
Wingra a	0.6	64	0.05	8
Wingra b	0.3	6	0	0
Wingra b	0.6	50	0.045	8
Monona Bay a	0.3	22	0.015	5
Monona Bay a	0.6	27	0.02	3
Monona Bay b	0.3	7	0	0
Monona Bay b	0.6	21	0.015	2
Waubesa a	0.3	63	0.05	2
Waubesa a	0.6	---	-----	--
Waubesa b	0.3	23	0.015	5
Waubesa b	0.6	---	-----	--
Kegonsa a	0.3	28	0.03	10
Kegonsa a	0.6	48	0.04	7
Kegonsa b	0.3	6	0	0
Kegonsa b	0.6	24	0.015	2

[a] Ave. algae dry-wt yield of duplicate cultures; 20-day culture period in Gorham's Medium major elements (-Fe) and vitamins B_1 and B_{12}.

Table 42. THE ABSORPTION OF PHOSPHORUS BY PHOSPHORUS-LIMITED *Cladophora* sp. FROM LAKE MICHIGAN

Sample[a]	Concentration of added PO_4-P, mg P/1	Extractable PO_4-P of algae, mg P/100 mg algae
A (Original)	----	0.057
B	0	0.043
C	0.16	0.12
D	0.24	0.20
E	0.32	0.18

[a] 10-15 mg *Cladophora* sp./250 ml Lake Michigan water; 14 hours sorption.

in Table 42. The extractable PO_4-P increased from 0.057 mg PO_4-P/100 mg for the original algae and from 0.043 for algae incubated without added PO_4-P to values as high as 0.20 with increasing amounts of added PO_4-P. Thus these algae were alive and capable of increasing their phosphorus content if more phosphorus had been available in the lake environment sampled.

Cladophora sp. collected from Lake Mendota (Madison, Wisconsin) during mid-August, 1973, were found to have high rates of NH_4-N absorption in the dark and were considered to be nitrogen-limited. This was substantiated by the observation that the algae were relatively free of epiphytes and had a pale yellow-green color (Fitzgerald, 1969). After samples of these algae were incubated overnight in Lake Mendota water fortified with different amounts of NO_3-N and NH_3-N, the rates of NH_4-N absorption were measured. The results are summarized in Table 43.

The NH_4-N absorption rates of the algae incubated with 0.8 and 3.2 mg NO_3-N/1 were only slightly reduced in comparison to algae incubated with no added nitrogen. When 13 mg NO_3-N/1 was present, the NH_4-N absorption rate was reduced to one-half; the same concentration of

Table 43. THE EFFECT OF AMMONIUM AND NITRATE NITROGEN ON THE NH_4-N ABSORPTION RATES OF NITROGEN-LIMITED *Cladophora* sp. FROM LAKE MENDOTA

Sample[a]	Nitrogen source	Concentration of added nitrogen, mg N/l	Rate of NH_4-N absorption, µg N/10 mg algae/hour
A	None	0	84
B	NO_3-N	0.8	64
C	NO_3-N	3.2	60
D	NO_3-N	13.0	32
E	NH_4-N	13.0	0

[a] 20-40 mg *Cladophora* sp./250 ml Lake Mendota water; incubated with shaking for 22 hours at 25°C.

Table 44. THE EFFECT OF INCUBATION WITH ADDED IRON ON THE IRON CONTENT OF *Cladophora* sp. FROM LAKE MENDOTA

Sample[a]	Concentration of iron,[b] mg Fe/l	Iron content of algae, % Fe
A	0	0.036
B	0.12	0.10
C	0.25	0.12
D	0.38	0.15
E	0.50	0.18
F	0.75	0.22
G	1.25	0.25
H	0	0.033

[a] 15-20 mg *Cladophora* sp./400 ml Lake Mendota water; incubated with aeration at 25°C.

[b] Fe-EDTA source of iron.

NH_4-N resulted in no absorption of NH_4-N by the algae. Therefore, if the Cladophora sp. from Lake Mendota had grown with adequate supplies of available nitrogen, it would have had relatively low NH_4-N absorption rates. Subsequent NH_4-N uptake tests after a rainfall in this area resulted in lower NH_4-N absorption rates and a change in color from pale yellow-green to dark green, presumably as a result of N in the rainfall.

Cladophora sp. with relatively low iron concentrations were collected from Lake Mendota during August and September, 1973. Samples of these algae were incubated in lake water and synthetic media to which a soluble form of iron had been added to determine if algae with low iron concentrations were capable of absorbing additional iron. The source of iron was Fe-EDTA, so there would be less chance of high iron concentrations in the algae due surface adsorption of insoluble iron precipitates. The results of one such experiment are summarized in Table 44. The iron concentration in the Cladophora sp. increased from 0.036 to 0.25% as the amount of iron added to the lake water increased, indicating the algae were capable of absorbing more iron if available.

SECTION X

EVALUATION OF NUTRIENT SUPPLIES IN THE GREAT LAKES FOR Cladophora sp. GROWTH

The results of evaluations of nutrient supplies and primary growth-limiting nutrients that might be critical in nuisance growths of Cladophora sp. in the Great Lakes are presented in this section. These evaluations were accomplished primarily by two bioassays, plant analysis and Fitzgerald tests.

EXPERIMENTAL PROCEDURES

The procedures used in culturing and analyzing C. glomerata to establish the base values for plant analysis and the Fitzgerald tests were described in Section VI and IX.

Samples of Cladophora sp. collected from lakes for analyses were thoroughly rinsed in water from the sampling area, squeezed between dry filter papers, and dried with forced warm air in a manner that permitted storage until chemical analyses were made.

GENERAL NUTRIENT EVALUATIONS BY PLANT ANALYSIS

Nutrient assay by plant analysis involves comparison of the critical concentration for each required inorganic nutrient in the aquatic plant under study with the concentrations of the same elements in field samples of the alga or macrophyte. A concentration of an element above the critical concentration indicates the element was in adequate supply at the time of sampling in the environment from which the sample was obtained. A concentration below the critical concentration indicates that available supplies of an element were inadequate for optimum growth. The element had become growth-limiting. Routine sampling of a body of water throughout the summer months will indicate whether a specific nutrient became growth-limiting at any time during the growing season.

A number of Cladophora sp. samples were collected from Lake Michigan. The locations of the sample sites, the dates of sampling, and the analyses for most of the essential elements are presented in Table 45.

The critical concentrations with which to compare the analytical data from field samples were presented in Table 23 in Section VI. Based on the critical concentrations presented in Table 23, there were no indications that N or P supplies were growth limiting at the sample points listed in Table 45. The critical N concentration was established as 1.1% in Section VI; the range in the field samples was from 1.71 to 4.30% with every sample except the one from Manastique on July 23, 1973, at least 100% in excess of the critical concentration. The critical P concentration established in Section VI was 0.06%; the range in P concentration was 0.17% to 0.86% critical concentration. The low P concentration of 0.17% also was well above the 0.10% critical P concentration established in another phase of the project (Section IX).

Concentrations of the essential major element cations Ca, Mg, and K also were far in excess of the critical concentrations of 0.30% Ca, 1.30% K, and 0.15% Mg. Again the lowest values were at least 2x the critical concentrations.

The high concentrations of Ca and Mg in the algae probably reflect the hard water and high concentrations of these two elements in Lake Michigan. The very high K concentrations in the algae are somewhat surprising. In three samples, K concentrations were in excess of 5.0 per cent, values comparable to concentrations in heavily fertilized agricultural crops.

The concentrations of Fe, Mn, and Zn in Lake Michigan _Cladophora_ sp. were far above the critical concentrations of 45 ppm Fe, 16 ppm Mn, and $\leq$ 5 ppm Zn. Very high Fe concentrations, in excess of 1000 ppm, may have been due to sample contamination with silt and sand particles or surface precipitation. There was no indication of a growth-limiting role for Fe, Mn, or Zn.

Cladophora sp. concentrations of two of the trace elements, Cu and B, were low enough to suggest they might have become or could become growth-limiting. The critical Cu concentration is $\leq$ 6.0 ppm. The Cu concentration in three algae samples was less than 6.0 ppm and in 10 of 20 samples the Cu concentration was 10 ppm or less. The critical

TABLE 45. INORGANIC ANALYSIS[a] OF Cladophora sp. COLLECTED FROM LAKE MICHIGAN AT VARIOUS SITES AND AT VARIOUS TIMES DURING THE SUMMER

Sampling area		Date	Element conc., %					Element conc., ppm				
			N	P	K	Ca	Mg	Fe	B	Cu	Zn	Mn
Michigan	Holland	10-18-73	3.35	0.38	3.10	3.31	0.31	2130	181	7.3	22	221
"	Manistique	7-23-73	1.71	0.17	6.60	1.17	0.21	1640	146	5.0	17	395
"	"	7-29-74	2.45	0.24	5.52	1.11	0.27	1920	106	5.4	66	329
Milwaukee, Wis.	N. Pt. Park	9-26-73	2.60	0.23	3.64	1.71	0.45	1660	219	8.0	31	120
"	"	10-14-73	3.66	0.43	2.85	1.25	0.49	2260	136	9.5	47	164
"	"	10-22-73	3.98	0.29	3.24	1.00	0.39	986	203	4.9	26	100
"	"	7-08-74	2.37	0.42	3.46	2.98	0.61	>2500	121	16.0	130	216
"	N. "	6-02-74	2.80	0.38	4.11	2.87	1.23	"	43	11.0	62	122
"	N. "	6-17-74	3.42	0.38	3.95	1.58	0.73	1580	76	7.2	73	86
"	S. "	6-02-74	--	0.24	5.37	1.15	0.47	923	34	6.5	45	62
"	S. "	6-17-74	3.99	0.29	4.67	0.82	0.30	869	73	7.1	57	46
"	1/2 mi. N. "	7-08-74	2.36	0.82	3.02	4.36	0.84	>2500	147	29.0	210	279
"	Bradford Beach	6-17-74	3.41	0.30	4.68	1.20	0.48	1420	95	7.1	60	79

" Harbor breakwater	Shore outside	6-17-74	3.69	0.27	4.79	0.87	0.31	755	96	5.1	36	41
" "	" inside	6-17-74	4.01	0.49	3.92	1.14	0.52	2340	114	160.0	93	197
" "	" outside	7-08-74	4.30	0.86	3.73	0.84	0.29	2500	135	12.0	140	183
" "	" inside	7-08-74	3.88	0.54	2.59	1.37	0.46	"	94	21.0	190	372
" "	1/2 mi. outside	7-08-74	3.88	0.34	3.99	1.03	0.31	877	90	7.8	83	90
" "	1/2 mi. inside	7-08-74	3.72	0.50	2.99	1.23	0.39	2500	92	15.1	200	358

[a] Total N analyses were by Micro-Kjeldahl procedure; all other analyses were with the Jarall-Ash Multichannel Spectrometer at the Wisconsin Alumni Research Foundation Laboratories, Madison, Wisconsin.

B concentration was established as 110 ppm, a value far in excess of the critical concentration in other plants, both agricultural and aquatic. In 9 of the 20 samples, the B concentration was below the critical concentration. In two samples from North Point Park in Milwaukee, B concentrations were only 43 and 34 ppm.

Analyses of Cladophora sp. from three other Great Lakes are presented in Table 46. For the most part the results are similar to the data from Lake Michigan. No N value was below the 1.1% critical concentration, although in two samples from Eastern Lake Erie N concentrations were 1.69 and 1.48%, only slightly above the critical concentration. Phosphorus concentrations were extremely high, averaging 0.49% in the 12 samples. Independence in the N and P concentrations is apparent. For example, Cladophora sp. samples from Eastern Lake Erie with the lowest N values were among the highest in total P concentration. One sample from Eastern Lake Erie (Geneva, Ohio) was very low in both Ca and Mg. Algae concentrations of K, Fe, and Mn were far above the critical concentrations.

No copper concentration was below the $\leq$ 6.0 ppm critical concentration, although four were below 10.0 ppm. In contrast to the Lake Michigan data, the Zn concentration in two samples from Lake Erie, Pt. Pelee Park and Geneva, Ohio, were below the critical concentration of 16 ppm. In common with the Lake Michigan data, the B concentration in two of the samples was below the critical concentration of 110 ppm. One sample was from Western Lake Erie; the other was from Lake Ontario.

Data on the concentrations in Cladophora sp. of several elements currently not considered essential for the growth of most plants are presented in Tables 47 and 48. The Jarrell-Ash Multichannel Emission Spectrometer routinely determines these elements. The elements are Na, Al, Ba, Sr, and Cr. At this time, the critical concentrations which would permit evaluation of these analyses have not been established. The data are made available for possible future use. It can be stated that except for Cr the Cladophora sp. data are comparable to values reported for economic crops (Chapman, 1966). In general,

TABLE 46. INORGANIC ANALYSIS[a] OF *Cladophora* sp. COLLECTED FROM LAKES ERIE, ONTARIO, AND HURON AT VARIOUS SITES AND AT VARIOUS TIMES DURING THE SUMMER

Sampling area		Date	Element conc., %[b]					Element conc., ppm[b]				
			N	P	K	Ca	Mg	Fe	B	Cu	Zn	Mn
Western												
L. Erie	Put-in-Bay, Ohio	8-29-73	2.19	0.34	3.82	2.71	0.28	>2500	148	13.0	33	207
	Rondeau Park, Ontario	10-20-73	2.79	0.46	3.84	2.29	0.36	>2500	230	7.8	23	651
	Pt. Pelee Park, Ont.	10-20-73	2.75	0.28	3.81	2.43	0.31	2230	256	6.1	14	120
	Port Clinton, Ohio	6-20-74	3.93	0.78	3.83	1.97	0.48	>2500	99	17.0	82	285
Eastern												
L. Erie	Geneva, Ohio	6-20-74	4.20	0.49	4.27	0.86	0.23	992	136	9.4	45	114
	Pt. Colbourne, Ontario	8-12-74	1.69	0.71	4.10	13.1	0.47	4120	247	32.0	49	450
	Welland Canal, Ontario	8-12-74	1.48	0.73	4.08	3.14	0.48	6680	306	24.0	46	368
L. Ontario	Olcott, N.Y., breakwater	8-20-73	2.72	0.33	3.56	4.59	0.25	1760	202	10.0	49	235
	Olcott, N.Y., beach rocks	6-20-74	4.40	0.57	4.00	0.59	0.18	721	70	8.9	52	91
	Olcott, N.Y., wharf	6-20-74	4.65	0.59	3.43	0.56	0.19	877	76	8.3	82	127
L. Huron	Pt. Sanilac, Michigan	10-19-73	2.30	0.26	3.05	4.32	0.39	>2500	247	9.5	41	623

[a] Total N analyses were by a micro-Kjeldahl procedure; all other analyses were with the Jarell-Ash Multichannel Spectrometer at the Wisconsin Alumni Research Foundation Laboratories, Madison, Wisconsin.

[b] The critical concentrations in *Cladophora* sp. are N, 1.1%; P, 0.66%; K, 1.30%; Ca, 0.30%; Mg, 0.15%; Fe, 45 ppm; B, 110 ppm; Cu, $\leq$6.0 ppm; Zn, $\leq$5.0 ppm; and Mn, 16 ppm.

TABLE 47. CONCENTRATIONS OF SEVERAL NON-ESSENTIAL ELEMENTS IN *Cladophora* sp. COLLECTED FROM LAKE MICHIGAN[a]

Sampling area		Date	Conc., %	Element conc., ppm			
			Na	Al	Ba	Sr	Cr
Michigan	Holland	10-18-73	0.07	801	23	66	15
Michigan	Manistique	7-23-73	0.03	655	14	57	5
Michigan	Manistique	7-29-74	0.05	698	17	58	6
Milwaukee, Wis.	N. Pt. Park	9-26-73	0.05	929	14	63	21
Milwaukee, Wis.	N. Pt. Park	10-14-73	0.06	1410	14	39	25
Milwaukee, Wis.	N. Pt. Park	10-22-73	0.04	447	10	57	12
Milwaukee, Wis.	N. Pt. Park	7-08-74	0.09	>3000	26	72	49
Milwaukee, Wis.	N., N. Pt. Park	6-02-74	0.09	1870	17	40	18
Milwaukee, Wis.	N., N. Pt. Park	6-17-74	0.07	846	11	31	15
Milwaukee, Wis.	S., N. Pt. Park	6-02-74	0.08	540	9	32	7
Milwaukee, Wis.	S., N. Pt. Park	6-17-74	0.05	471	9	37	9
Milwaukee, Wis.	N., N. Pt. Park	7-08-74	0.14	>3000	29	85	43
Milwaukee, Wis.	Bradford Beach	6-17-74	0.05	916	11	31	17

Milw., breakwater	Shore outside	6-17-74	0.05	409	8	32	9
Milw., breakwater	Shore inside	6-17-74	0.07	2020	22	44	28
Milw., breakwater	Shore outside	7-08-74	0.07	416	9	61	13
Milw., breakwater	Shore inside	7-08-74	0.11	2780	23	52	85
Milw., breakwater	1/2 mi. outside	7-08-74	0.06	577	10	44	12
Milw., breakwater	1/2 mi. inside	7-03-74	0.10	1990	20	51	78

[a] Analyses were with the Jarell-Ash Multichannel Spectrometer at the Wisconsin Alumni Research Foundation Laboratories, Madison, Wisconsin.

TABLE 48. CONCENTRATIONS OF SEVERAL NON-ESSENTIAL ELEMENTS IN _Cladophora_ sp. COLLECTED FROM LAKES ERIE, ONTARIO, AND HURON[a]

Sampling area		Date	Conc., %	Element conc., ppm			
			Na	Al	Ba	Sr	Cr
Western							
L. Erie	Put-in-Bay, Ohio	8-29-73	0.09	2430	22	76	29
	Rondeau Park, Ontario	10-20-73	0.07	2680	34	78	11
	Pt. Pelee Park, Ontario	10-20-73	0.06	928	14	66	8
	Port Clinton, Ohio	6-20-74	0.10	>3000	40	105	16
Eastern							
L. Erie	Geneva, Ohio	6-20-74	0.08	852	11	43	7
	Pt. Colbourne, Ontario	8-12-74	0.24	4570	64	172	48
	Welland Canal, Ontario	8-12-74	0.17	7180	63	148	29
L. Ontario	Olcott, N.Y., breakwater	8-20-73	0.11	1310	23	96	18
	Olcott, N.Y., beach rocks	6-20-74	0.07	230	8	28	8
	Olcott, N.Y., wharf	6-20-74	0.05	428	9	39	10
L. Huron	Pt. Sanilac, Michigan	10-19-73	0.05	2540	30	86	13

[a] Analyses were with the Jarell-Ash Multichannel Spectrometer at the Wisconsin Alumni Research Foundation Laboratories, Madison, Wisconsin.

the lowest Cr concentrations in Cladophora sp. were comparable to high values in economic plants. Very high Al concentrations correlate with high Fe concentrations, probably because both reflect soil contamination of samples.

ADDITIONAL EVALUATIONS OF P SUPPLIES

Because of the key role assigned P in promoting nuisance aquatic plant growths and the emphasis placed on P removal to reduce these growths, additional bioassays and evaluations were carried out on P availability in Great Lakes samples. The P data in the previous section obtained with a Jarrell-Ash Emission Spectrometer are not as reliable as data obtained by the best quantitative procedures. For that reason P analyses in this section were by standard colorimetric procedures. Some data on Fitzgerald tests for NH_4-N uptake as a measure of N availability to Cladophora sp. and on Fe concentrations as a measure of Fe availability are included.

Lake Superior and Northern Lakes Michigan and Huron

Algae samples were collected at several points on the north shore of Lake Superior on two trips during the summer of 1974. Most of the north shore is free of readily collectable algae. Cladophora sp. were only found in the harbor area at Duluth. This could be due to the relatively warm water temperature in this enclosed area compared to the open lake. The most common alga found in different sampling areas was a species of Ulothrix, perhaps because of relatively low water temperatures in Lake Superior even in midsummer. The growth of Ulothrix sp. usually precedes the growth of Cladophora sp. as lake temperatures increase. A summary of the concentrations of the total P and Fe in algae from Lake Superior is presented in Table 49.

The relatively high concentrations of P in several species of filamentous green algae from the harbor area of Duluth (0.38-0.54%) indicates there are readily available sources of P in this area. In contrast, samples of Ulothrix sp. from the northern outskirts of Duluth (Lester River outlet) contained less than half as much P (0.14-0.17%). Relatively low concentrations of P also were found

TABLE 49. TOTAL P AND TOTAL Fe ANALYSES OF SEVERAL ALGAE SPECIES COLLECTED IN LAKE SUPERIOR AND IN NORTHERN LAKES MICHIGAN AND HURON

Sampling date	Sampling area	Algae	Total P, %	Total Fe, %
7-22-74	L. Superior; Duluth, Mn., harbor	*Cladophora*	0.40	0.11
7-22-74	L. Superior; Duluth, Mn., harbor	*Ulothrix*	0.38	0.10
8-25-74	L. Superior; Duluth, Mn., harbor	*Cladophora*	0.54	0.10
8-25-74	L. Superior; Duluth, Mn., harbor	*Hydrodictyon*	0.44	0.24
7-24-74	L. Superior; Duluth, Mn., Lester R. outlet	*Ulothrix*	0.17	0.11
8-25-74	L. Superior; Duluth, Mn., Lester R. outlet	*Ulothrix*	0.14	0.18
7-24-74	L. Superior; Tofte, Mi., beach	*Ulothrix*	0.68	0.38
8-25-74	L. Superior; Tofte, Mi., beach	*Ulothrix*	0.70	0.37
7-23-74	L. Superior; Grand Marais, Mi.	*Ulothrix*	0.076	0.058
8-24-74	L. Superior; Grand Marais, Mi.	*Ulothrix*	0.10	0.19
8-26-74	L. Superior; Grand Marais, Mi.	*Ulothrix*	0.071	0.093
7-23-74	L. Superior; Thunder Bay, Ont., canal	Diatoms	0.10	0.42
8-25-74	L. Superior; Thunder Bay, Ont., canal	Blue-green	0.52	0.81
7-26-74	L. Mich.; Manistique, Mi., E. breakwater	*Cladophora*	0.11	0.21
7-26-74	L. Mich.; Manistique, Mi., E. breakwater	*Ulothrix*	0.10	0.21

8-14-74	L. Mich.; Manistique, Mi., E. breakwater	*Cladophora*	0.14	0.28
8-14-74	L. Mich.; Manistique, Mi., E. breakwater	*Ulothrix*	0.16	0.74
8-14-74	L. Mich.; Manistique, Mi., W. breakwater	*Ulothrix*	0.19	0.42
9-12-74	L. Mich.; Manistique, Mi., E. breakwater	*Cladophora*	0.13	0.33
9-12-74	L. Mich.; Manistique, Mi., W. breakwater	*Cladophora*	0.10	0.47
9-12-74	L. Mich.; Manistique, Mi., W. breakwater	*Ulothrix*	0.088	0.66
7-26-74	L. Mich.; Petoskey, Mi., beach	*Cladophora*	0.24	0.071
7-26-74	L. Huron; St. Ignace, Mi., canal	*Cladophora*	0.40	0.58
7-26-74	L. Huron; Mackinaw City, Mi.	*Cladophora*	0.11	0.075
7-26-74	L. Huron; Mackinaw City, Mi.	*Cladophora*	0.10	0.034

in Ulothrix sp. from the harbor at Grand Marais, Michigan, (0.07 - 0.10%) and in diatoms from the harbor at Thunder Bay, Ontario (0.10%). Ulothrix sp. from near Tofte, Michigan, contained the highest concentrations of P (0.68-0.70%) found in any of the Lake Superior samples, perhaps due to input of sewage from a small nearby community. These results of P analyses of in situ algae agree with the data and conclusions of Plumb and Lee (1974) using other methods for measuring nutrient availability in this area of Lake Superior.

Duluth Harbor does not appear to be a relatively important source of available Fe for Lake Superior, since Fe concentrations in Ulothrix sp. from the harbor area and the open lake areas (Table 49) were approximately the same (0.10-0.18% Fe). Iron concentrations in Ulothrix sp. from near Tofte, Michigan, were exceptionally high (0.37-0.38% Fe). It would seem worthwhile to study this north shore area of Lake Superior to determine if Fe entering the lake from different mining areas is available to algae without the addition of chelation chemicals that can be contributed by municipal sewage effluents.

Phosphorus concentrations in Cladophora sp. from northern Lakes Michigan and Huron were relatively low (0.088-0.19%). In contrast, P concentrations in algae from Petosky and St. Ignace, Michigan, were relatively high (0.24-0.40%) probably due to local pollution.

Iron concentrations in algae collected from the Manistique, Michigan, area of Lake Michigan were high (0.21-0.66% Fe) in comparison to Fe concentrations in other samples from northern Lakes Michigan and Huron (0.034-0.075% Fe). The high concentrations of Fe in algae from Manistique were expected since the reddish color of rivers in this area is associated with dissolved and suspended iron compounds derived from iron ore in the area.

Milwaukee Area of Lake Michigan

The total P and Fe concentrations in Cladophora sp. collected from the Milwaukee area during the summer of 1974 are presented in Table 50. The data in general indicate that P concentrations in algae

TABLE 50. TOTAL P AND Fe ANALYSES OF *Cladophora* sp. COLLECTED FROM THE MILWAUKEE AREA OF LAKE MICHIGAN IN 1974

Sampling date	Sampling area	Total P,[a] %	Total Fe,[a] %
6-17-74	North Point Park, North	0.29	0.098
6-17-74	North Point Park, South	.29	.080
7-08-74	North Point Park, North	.12	.24
7-08-74	North Point Park, South	.11	.18
7-15-74	North Point Park, North	.15	.18
7-15-74	North Point Park, South	.14	.40
10-21-74	North Point Park, North	.12	.12
10-21-74	North Point Park, South	.16	.10
6-17-74	Bradford Beach	.25	.09
7-16-74	Bradford Beach	.16	.31
10-21-74	Bradford Beach	.14	.11
6-17-74	Breakwater, outer side	.26	.080
6-17-74	Breakwater, inner side	.39	.16
7-08-74	Breakwater, outer side	.22	.24
7-08-74	Breakwater, inner side	.38	.56
7-16-74	Breakwater, outer side	.23	.084
7-16-74	Breakwater, inner side	.38	.31
10-21-74	Breakwater, outer side	.14	.11
10-21-74	Breakwater, inner side	.20	.093

[a] Data reported are averages of duplicate analysis.

collected from outside the Milwaukee Harbor (North Point Park, Bradford Beach, and Outer side of breakwater) were considerably lower than in algae collected within the harbor (Inner side of breakwater). Average total P values were 0.19% and 0.34%, respectively, for the two areas. The low concentrations of P in algae from areas outside the harbor and to the north (North Point Park) indicate that, whereas the Milwaukee Harbor can be a source of P for the algae within the harbor, it does not provide surplus P for algae outside the harbor area.

The concentration of Fe in the Cladophora sp. from the harbor area varied considerably between the different sampling dates and sites, but in general samples from within the harbor area had higher Fe concentrations than samples from outside the harbor.

Fitzgerald tests on NH_4-N uptake, extractable PO_4-P, and total Fe concentration in several algae samples collected outside the Milwaukee harbor in 1973 are presented in Table 51. Two of the three samples were indicated to be N-deficient by NH_4-N uptake values in excess of 15 μg N/10 mg algae/hr. All three samples were from algae indicated to be deficient or close to deficient in P by comparisons with the critical extractable PO_4-P concentration of 0.08%. There was no indication that the algae were deficient in Fe.

Lake Erie Collections

Total P and Fe analyses of Cladophora sp. from Lake Erie (Tables 52 and 53) show that P concentrations in algae from western Lake Erie (average, 0.34%) are considerably higher than in algae from the eastern part of the lake (average, 0.089%). An exception is the two samples from the northwestern side of Lake Erie (Ontario, Canada) which had comparatively low P concentrations of 0.13 and 0.20%. Algae with high P concentrations (0.22-0.56%) were collected from the Put-in-Bay area to Cleveland, Ohio, which would indicate that the entire area of the southwestern shore of Lake Erie has excessive amounts of available P for algae growth. These data agree with the report by Glooschenko and Moore (1972) which stated that primary productivity in the western basin of Lake Erie was much higher than in the central or eastern basins.

The fact that P concentrations in Cladophora sp. from the eastern area of Lake Erie during August, 1974, indicated the algae were P-limited or nearly P-limited has considerable ecological significance. These algae were P-limited at a time when Cladophora sp. from the western basin of the lake contained surplus P. Therefore, the lake muds of the eastern part of Lake Erie do not provide adequate available P during

TABLE 51. FITZGERALD TESTS FOR N, P, AND Fe SUPPLIES TO Cladophora sp. COLLECTED FROM THE MILWAUKEE HARBOR AREA OF LAKE MICHIGAN, 1973

Sampling date	Sampling area	NH_4-N absorption, N/10 mg/hr	Extract PO_4-P,[a] %	Total Fe,[a] %
8-17-73	North Point Park	30	0.084	0.046
8-17-73	End of breakwater	32	0.10	0.033
9-26-73	North Point Park	4	0.057	0.075

[a] Data reported are the averages of triplicate analyses.

late summer to meet algae demands and cannot be considered an inexhaustible P source. As a result, reduction in local additions of P, such as effluents from municipal and industrial sewage plants should aid in the further control of Cladophora sp. in this part of Lake Erie. In developing measures to control the algae, more detailed algae sampling of eastern Lake Erie should be carried out to determine the seasonal cycle of nutrient levels in the algae and to locate significant nutrient sources.

Lake Ontario

The few analyses of the nutrient content of Cladophora sp. from the Olcott, N.Y., area of Lake Ontario (Table 54) indicate these algae have high concentrations of P (0.58-0.66%), but relatively low concentrations of Fe (0.061-0.10% Fe). More detailed studies should be carried out to determine the seasonal cycle of nutrients in the algae of this and other areas of Lake Ontario, which would permit evaluation of the significant sources of nutrients for the algae. Studies of other deep fertile lakes have shown that despite significant contributions of nutrients by spring and fall lake turnover, some of these lakes become depleted of N, P, and Fe during midsummer. Thus, midsummer pollution can have a significant influence on midsummer algae growth (Fitzgerald and Uttormark, 1974).

TABLE 52. TOTAL P AND Fe CONCENTRATIONS IN Cladophora sp. COLLECTED FROM WESTERN LAKE ERIE

Sampling date	Sampling area	Total P, %	Total Fe, %
10-20-73	Rondeau Park, Ont.	0.13	0.17
10-20-73	Pt. Pelee Park, Ont.	0.20	0.07
5-28-74	Put-in-Bay, Oh., E. boat docks	0.42	0.076
5-28-74	Put-in-Bay, Oh., W. boat docks	0.56	0.039
6-26-74	Pt. Clinton, Oh., breakwater	0.46	0.28
9-04-75	Avon Park, Oh., W. of Cleveland	0.46	0.24
9-04-74	Sheffield, Oh., W. of Cleveland	0.24	0.12
9-04-74	Beaver Park, Oh., E. of Vermillion	0.41	0.26
9-04-74	Sandusky Bay, Oh.	0.28	0.38
9-04-74	Danbury, Oh., E. of Pt. Clinton	0.22	0.40
9-04-74	Port Clinton, Oh., E. of boat docks	0.35	0.13
9-04-74	Port Clinton, Oh., W. of boat docks	0.25	0.074
9-04-74	Port Clinton, Oh., Put-in-Bay docks	0.43	0.068
5-17-75	Port Clinton, Oh., breakwater	0.36	0.42
5-18-75	Put-in-Bay, Oh., W. of boat docks	0.40	0.48

TABLE 53. TOTAL P AND Fe CONCENTRATIONS IN Cladophora sp. COLLECTED FROM EASTERN LAKE ERIE

Sampling date	Sampling area	Total P, %	Total Fe, %
8-11-74	Fort Erie, Ont., river wall	0.070	0.063
8-12-74	Port Colbourne, Ont., shore W. of city	0.13	0.36
8-12-74	Port Colbourne, Ont., shore W. of city	0.088	0.14
8-12-74	Port Colbourne, Ont., shore W. of city	0.070	0.18
8-12-74	Port Colbourne, Ont., harbor	0.14	0.076
8-12-74	Port Colbourne, Welland Canal, swimming area	0.10	0.050
8-12-74	Welland, Ont., Welland Canal	0.068	0.056
8-12-74	Allanburg, Ont., Welland Canal, W. shore	0.065	0.18
8-12-74	Allanburg, Ont., Welland Canal, E. shore	0.078	0.12
5-18-75	Fort Erie, Ont., N. river wall	0.22	0.14
5-18-75	Fort Erie, Ont., central river wall	0.17	0.11
5-18-75	Fort Erie, Ont., S. river wall	0.10	0.026

TABLE 54. TOTAL P AND Fe CONCENTRATIONS IN Cladophora sp. COLLECTED FROM LAKE ONTARIO

Sampling date	Sampling area	Total P, %	Total Fe, %
8-30-73	Olcott, N.Y., breakwater	--	0.066
5-23-74	Olcott, N.Y., breakwater	0.63	0.10
5-23-74	Olcott, N.Y., beach rocks	0.66	0.061
6-27-74	Olcott, N.Y., breakwater	0.58	0.068
6-27-74	Olcott, N.Y., beach rocks	0.58	0.086

Green Bay, Lake Michigan

Concern over pollution of Green Bay, Lake Michigan, from heavy industrial and municipal waste loadings along the Fox River led to a study of effects of these pollutants on the nutrition of Cladophora sp. from the area. The sources of pollution in Green Bay and the degree of pollution in various parts were correlated with nutrient concentrations in algae from different parts of the study area.

Sources of Nutrients

The major tributary to Green Bay, the Fox River, flows into the extreme southern end of the bay (Figure 8). It is severely polluted because of the heavy waste loadings contributed by industry and municipalities (approx. 275,000 population). Because of its large input volume, the river has a significant influence on the water quality and biota of the bay.

Sources of P and N to the Lower Fox River, the only section of the river subjected to severe deterioration, are enumerated in Table 55. (Epstein, et al., 1974; Sager and Wiersma, 1972; Schraufnagel et al., 1968; Shridharan, 1972). On an annual basis, Lake Winnebago provides about 85% of the total N added to the Lower Fox River; municipal sewage treatment effluents and effluents from the pulp and paper industry add another 24,000 lbs N/day, or 13% of the total. Dairy

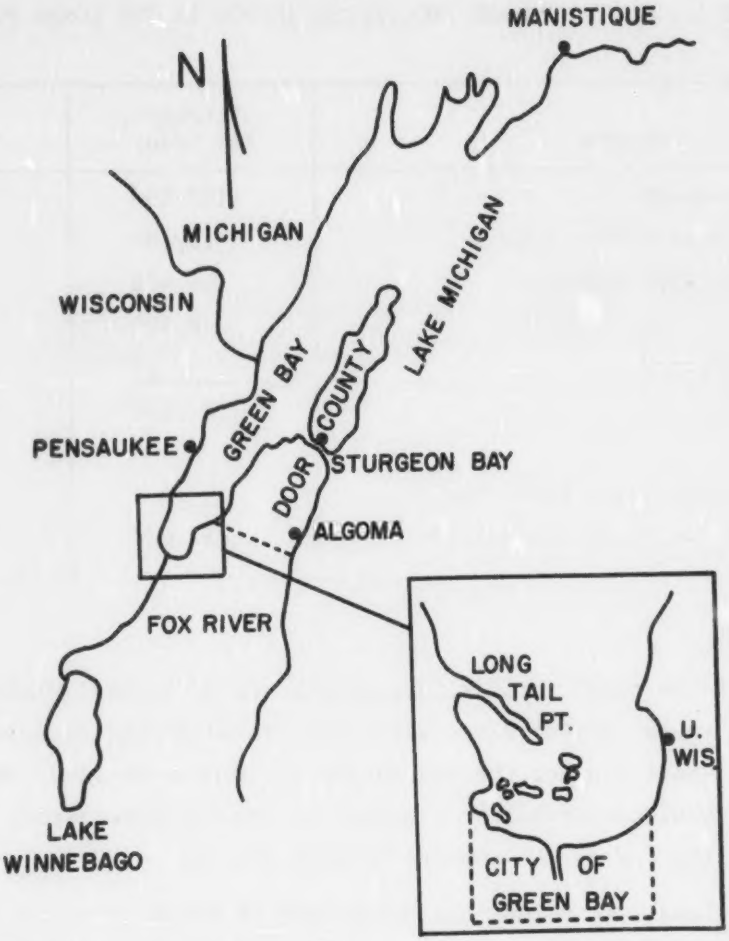
MANISTIQUE
N
MICHIGAN
WISCONSIN
LAKE MICHIGAN
GREEN BAY
DOOR COUNTY
PENSAUKEE
STURGEON BAY
ALGOMA
FOX RIVER
LAKE WINNEBAGO
LONG TAIL PT.
U. WIS
CITY OF GREEN BAY

Figure 8. Green Bay and Lake Michigan area.

and other industries contribute the remaining 2%. In contrast to N, the input of P from Lake Winnebago to the Lower Fox nearly equals the phosphorus added by the municipal treatment plants which line the river. Pulp and paper plants contribute about 1,100 lbs P/day, or half the amount contributed either by Lake Winnebago or by the municipal plants. Other industrial sources of P are negligible. South of Long Tail Point (Figure 8), Fox River water constitutes

TABLE 55. NITROGEN AND PHOSPHORUS INPUTS TO THE LOWER FOX RIVER

Source	Nitrogen, lbs N/day	Phosphorus, lbs P/day
Lake Winnebago	153,000	2,160
Municiple treatment plants	10,400	2,200
Pulp and paper mills	13,400	1,100
Dairy	4,300	--
Other industry	16	16
Total	181,116	5,476
Nutrients entering Green Bay at the Fox River (annual ave.)	232,000	4,310

50-80% of the total water volume present in Green Bay. Under southerly winds, a tongue of Fox River water was traced northward 9.3-12.4 mi along the east side of the bay during the summer of 1969. Modlin and Beeton (1970) confirmed the presence of the Fox River along the eastern shore of the bay in the summers of 1968 and 1969.

North of Long Tail Point, the proportion of Fox River water in Green Bay decreases rapidly. More than 15.5 miles north of the river mouth, values exceeding 25% of the total volume attributable to the Fox are seldom observed (Ahrnsbrak and Ragotzkie, 1970). Still farther north, the effects of the Fox River are minimal with respect to water volume and chemistry.

Sager and Wiersma (1972) recorded high concentrations of orthophosphate south of the Grassy Islands during the summer of 1971. The concentration gradient in the first few miles from the mouth of the Fox River was considered steep (from 33 to less than 8 μg P/l in 5 mi). Generally, concentrations along the east half of the bay were higher than in the west. These lower values illustrate the dispersal of Fox River water following entry into Green Bay.

Rousar and Beeton (1973) measured total P in transects across lower Green Bay in the summer of 1971. Phosphorus concentrations decreased with increasing distance from the mouth of the Fox River. Inshore concentrations exceeded offshore concentrations. Within a transect, the highest values for total P were generally found along the eastern shore of the bay.

Results

Seventy-one samples of Cladophora sp. were collected on three sampling dates during September, 1974, from shoreline stations in Green Bay and nearby Lake Michigan. For comparative purposes, some Cladophora sp. samples also were collected from other areas of the Great Lakes during July and August, 1974.

The results of triplicate analyses of the Cladophora sp. samples from each station for P, N, and Fe are summarized in Table 56. The plot (Figure 9) of P concentrations in the Cladophora sp. samples compares the analyses from the different areas sampled.

Phosphorus concentrations in the Cladophora sp. from along the east shore of the lower bay were relatively high, decreasing from 0.35% P for the bright green algae in the dense stands near the University of Wisconsin-Green Bay campus to 0.20% P at sampling sites 10 to 20 miles farther north. The total N and Fe concentrations also decreased with increasing distance from the Fox River outlet. However, only N decreased to values considered close to or below the critical nutrient concentration.

All samples collected 30 miles or more north of the Fox River outlet on the shores of the Door County peninsula (areas B and C) were relatively low in P, were usually from sparse growths, and were of a yellowish-green color. Algae in these areas also had relatively low Fe content, especially those samples collected from area C on the northern part of the peninsula. The samples from area D on Lake Michigan illustrate the effects small rivers and cities (Algoma) can have on the P concentration of local stands of Cladophora. At the river outlet in Algoma, Cladophora sp. growing on piers and

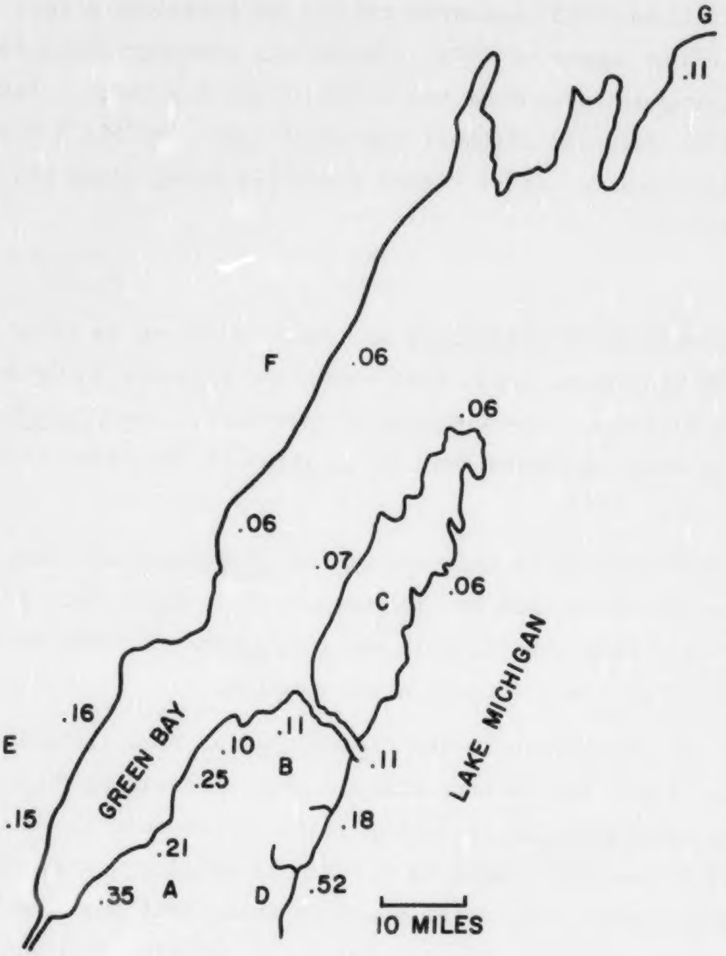
G
.11
F
.06
.06
.06
.07
C
.06
LAKE MICHIGAN
E
.16
GREEN BAY
.10
.11
B
.11
.25
.15
.18
.21
.35
A
D
.52
10 MILES

Figure 9. Green Bay and Lake Michigan Sampling Area and Total P (%) of Cladophora sp.

rocks was especially thick and dark green, and the P concentrations were very high at 0.52%. The Fe content, however, was not much different from that of Cladophora sp. growing along the northern part of the peninsula.

The P concentrations in algae collected along the western shore of Green Bay were low compared with concentrations in algae from the eastern part of the bay, as would be expected if the flow of the

TABLE 56. SUMMARY OF ANALYSES OF *Cladophora* sp. COLLECTED DURING SEPTEMBER, 1974, FROM GREEN BAY AND LAKE MICHIGAN

Sampling area	Average of triplicate analyses, %		
	Total P	Total N	Total Fe
Area A			
Campus, U. of Wi.	0.35	3.8	0.18
Bay Shore Park	0.21	2.9	0.14
Sugar Creek	0.25	1.4	0.11
Area B			
Claflin Park	0.10	1.9	0.085
Potawatomi State Park	0.11	-	0.11
Sturgeon Bay Outlet	0.11	2.4	0.11
Area C			
Egg Harbor, Wi.	0.069	1.8	0.051
Gill's Point	0.062	-	0.053
Baileys Harbor, Wi.	0.057	2.1	0.038
Area D			
Clay Banks	0.18	-	0.060
Algoma, Wi.	0.52	-	0.056
Area E			
Little Suamico, Wi.	0.15	-	0.20
Brookside, Wi.	0.16	2.9	0.15
Area F			
Menominee, Mi.	0.063	2.2	0.14
J. W. Wells State Park	0.058	-	0.20
Area G			
Manistique, Mi.	0.11	2.6	0.48

Fox River is mainly along the eastern shore.

The general conclusion from this study was that the growth of algae seems generally to be limited by available P during the algal growing season, except in polluted areas. This corroborates the work of Schelske *et al.* (1974) which also indicated that P limits phytoplankton growth in Lake Michigan.

Despite an annual average contribution of more than two tons of P per day to Green Bay by the Fox River, the *Cladophora* sp. within 30 miles of the river outlet are P-limited during late summer. Therefore, in September and November, Green Bay does not supply Lake Michigan with major quantities of algal nutrients that can enhance algal growth. If Green Bay can effectively purify itself, then other areas of the Great Lakes may do so also. A combination of tertiary treatment of wastes and the ability of Green Bay to purify itself should result in a marked quantitative improvement in the algal crop of the lower Green Bay area.

DISCUSSION

The studies described in this section were concerned primarily with evaluating nutrient supplies and identifying growth-limiting nutrients for *Cladophora* sp. in the Great Lakes. These are the principal practical applications of the project. Information on growth-limiting nutrients and nutrient supplies should be valuable in developing plans and measures for reducing nuisance algae growths.

Several aspects of the results justify additional comment. First, the results suggest that the development and use of plant analysis bioassays for evaluating nutrient supplies is worthwhile and to be recommended. This is supported by close correlations between plant bioassay evaluations of N and P supplies in several areas of the Great Lakes and the recognized N and P pollution of those areas.

The best example of such a correlation was the degree to which the total P in *Cladophora* sp. from Green Bay decreased as the distance of the points of sample collection from the point of discharge of effluent from the Fox River increased. The plant analysis bioassay also offers an advantage in making possible evaluations of supplies of inorganic nutrients other than N and P. This includes the essential trace elements. Because the base data on water concentrations which represent adequate and potentially deficient supplies of elements other than N and P are not available at this time, chemical analyses of water samples for elements other than N and P are difficult to interpret in terms of potential for plant growth.

Of the plant bioassay data, the results on the N and P status of Cladophora sp. probably are of most interest, because of the general recognition of the critical role of these elements in nuisance algae growths and the possibilities for control measures oriented toward these elements, particularly P. There was little indication that N supplies were reduced to levels that limited Cladophora sp. Nitrogen concentrations in the algae usually were well in excess of the 1.1% critical concentration. However, P concentrations in the Cladophora sp. not only correlated well with the general pollution of areas, but also in samples from relatively unpolluted areas approached the critical P concentrations, both total P and extractable PO_4-P. Low P concentrations were observed in some Cladophora sp. samples from northern Lakes Michigan and Huron, from Lake Michigan near the Milwaukee Harbor, from the upper parts of Green Bay, and from Eastern Lake Erie. In contrast, most samples from Western Lake Erie, lower Green Bay, and Lake Ontario were very high in P. Although positive recommendations probably should be based on more data, the results suggest that reduction in P supplies would be a practical means of reducing nuisance growths of Cladophora sp. in the Great Lakes.

The assays by plant analysis for supplies of elements other than N and P are interesting because they suggest that elements other than N and P actually become limiting for Cladophora sp. growth in the Great Lakes. This was true of Zn and particularly of B. The practical value of these observations cannot be assessed until further samples are taken and analyzed. A potential growth-limiting role for elements other than N and P also was indicated in a Lake Michigan water enrichment experiment reported in Section VII. In addition, relatively little Cladophora sp. growth developed in Lake Michigan water unless vitamin B_1 was added. Vitamin B_1, therefore, also is a potentially growth-limiting nutrient in the Great Lakes. Again, further studies are needed to establish the significance of this observation.

Definite and reliable critical concentrations are essential in successful applications of the plant analysis bioassay. The considerable difference in the critical concentration for P established in independent efforts by the two Principal Investigators in different aspects of this project (Sections VI and IX) indicates there can be difficulties in obtaining and interpreting the data from which critical concentrations are derived. Additional studies are necessary to establish the critical concentration of P and other key elements more precisely. It is possible that the critical concentration of P, and other elements as well, varies somewhat with the age of the Cladophora glomerata, as a result of changes in non-protoplasmic constituents and food reserves. The 0.08% critical value for hot water extractable P in the Fitzgerald test seems too high. It should not exceed the critical concentration based on total P in the algae cells. Again additional calibration tests are required. The data do seem to justify the view that, compared with other algae, the critical concentrations of N and P are relatively low for Cladophora glomerata, 0.06-0.1% for total P and 1.1-1.5% for total N.

SECTION XI

RELIABILITY OF SAMPLING AND ANALYTICAL METHODS

To attach ecological significance to differences in the inorganic composition of algae from various environments, the variability associated with the sampling procedure and with the analytical procedures should be known. Tests were carried out to evaluate both aspects of experimental variability.

RESULTS AND DISCUSSION

Replicate Analyses of Cladophora sp. Samples

A sample of Cladophora sp. was collected from Lake Monona and from Lake Wingra, Madison, Wisconsin, and total P and extractable PO_4-P analyses were made on ten replicate aliquots from each sample. The range in variation and the standard deviation for the analyses of each sample are presented in Table 57. The mean P concentration in the Lake Monona sample was considerably higher than in the Lake Wingra algae. In fact, the data would be interpreted to indicate the supply of P in Lake Monona was adequate for Cladophora sp. growth but P was limiting and inadequate for the Lake Wingra algae. The agreement in the replicate analyses in general is equal to the agreement obtained in routine use of the best quantitative procedures in plant analyses. Also, the data indicate that the analytical procedures employed are suitable for distinguishing by plant bioassay algae adequately supplied and deficient in P.

Two separate laboratories have been involved in this project. To further evaluate the reliability of the analytical procedures used, replicated analyses for P, Fe, N, and Ca were made on aliquots from samples collected from Lakes Monona and Wingra on July 11, 1974. The analyses included a comparison of results obtained by the separate laboratories involved in this project. The P method used in the Algae Laboratory is an acid-persulfate digestion (autoclave) followed by stannous chloride color development (Standard Methods, 13th Edition); the procedure used in the Botany Department Laboratory involved dry-ashing at 600°C followed by development of a

Table 57. AGREEMENT IN REPLICATE ANALYSES FOR TOTAL AND EXTRACTABLE P IN FIELD SAMPLES OF *Cladophora* sp.

	L. Monona		L. Wingra	
	Extract PO_4-P, %	Total P, %	Extract PO_4-P, %	Total P, %
Replicates, %	0.16	0.33	0.018	0.056
	0.16	0.36	0.016	0.066
	0.17	0.38	0.017	0.056
	0.17	0.36	0.018	0.058
	0.18	0.39	0.015	0.062
	0.16	0.44	0.013	0.061
	0.16	0.40	0.015	0.061
	0.18	0.41	0.017	0.064
	0.18	0.35	0.030	0.065
	0.19	0.33	0.018	0.065
Ave., %	0.17	0.38	0.018	0.061
Range, %	0.16-.19	0.33-.44	0.013-.030	0.056-.066
S.D.	0.01	0.04	0.005	0.004

vanadomolybdate complex which is yellow in color (Jackson, 1958). The results of the analyses are presented in Table 58. Again the data indicate that the agreement among replicates equals that expected from procedures in general use for plant analyses. This included total P analyses by the slightly different procedures used in the two participating laboratories.

Replicated Sampling of *Cladophora* sp.

Tests were carried out to measure the variability in the concentrations of several nutrients in *Cladophora* sp. collected from within a small area in a lake. This information is important in determining whether differences in nutrient concentrations in algae truly reflect variations in environmental nutrient supplies or merely normal

Table 58. AGREEMENT IN REPLICATE ANALYSES FOR P, N, Ca, AND Fe IN SAMPLES OF *Cladophora* sp.

Sampling area	Analysis and lab	Replicates, %						Ave., %	Range, %	S.D.
L. Mendota	Total P, Algae lab.	0.42	0.37	0.43	0.38	0.40	0.42	0.40	0.37-.43	0.02
L. Mendota	Total P, Bot. lab.	0.46	0.42	0.43	0.43	0.42	0.41	0.43	0.41-.46	0.02
L. Mendota	Total Fe, Algae lab.	0.071	0.066	0.055	0.078	0.074	0.069	0.069	0.055-.074	0.008
L. Mendota	Total N, Bot. lab.	4.35	4.20	4.22	4.28	4.54	-----	4.32	4.20-4.54	0.14
L. Mendota	Total Ca, Bot. lab.	0.70	0.70	0.73	0.70	0.70	0.71	0.71	0.70-0.73	0.01
L. Wingra	Total P, Algae lab.	0.28	0.28	0.24	0.27	0.28	0.26	0.27	0.24-.28	0.02
L. Wingra	Total P, Bot. lab.	0.22	0.24	0.23	0.23	0.23	0.24	0.23	0.22-.24	0.01
L. Wingra	Total Fe, Algae lab.	0.13	0.12	0.13	0.15	0.14	0.15	0.14	0.12-.15	0.01
L. Wingra	Total N, Bot. lab.	2.29	2.54	2.49	2.47	2.65	-----	2.49	2.29-2.65	0.13
L. Wingra	Total Ca, Bot. lab.	1.57	1.62	1.70	1.84	1.65	1.62	1.67	1.57-1.84	0.10

biological variation in the algae.

Ten samples of Cladophora sp. were collected from within an area 5 feet in radius within the breakwater of the Milwaukee Harbor; 10 samples were obtained at North Point Park, about one-half mile north of the harbor; and six samples were collected at Put-in-Bay, Lake Erie. The results of analyses of these samples for total P and Fe are presented in Table 59. The results indicate a high degree of reproducibility in the samples, particularly in the total P analyses. The 0.33-0.44% range in P concentration in the Milwaukee Harbor samples was the same as the range in the replicate analyses of the algae collected from Lake Monona and reported in Table 57. In other words, the analytical procedure could have been solely responsible for the differences observed. There was considerable variation in the total Fe concentration in the North Point Park samples, perhaps because the concentrations were relatively low. In general, the high degree of reproducibility in analyses of Cladophora sp. samples from within small areas supports the concept that variations in the concentration of an element in the algae do reflect variations in environmental supplies.

Table 59. AGREEMENT IN THE TOTAL P AND TOTAL Fe CONCENTRATIONS IN SAMPLES OF *Cladophora* sp. COLLECTED FROM WITHIN A FIVE-FOOT RADIUS

	L. Mich. Milw., Wis. breakwater		L. Mich. Milw., Wis. N. Pt. Park		L. Erie Put-in-Bay, Ohio	
	Total P, %	Total Fe, %	Total P, %	Total Fe, %	Total P, %	Total Fe, %
	0.44	0.28	0.16	0.04	0.39	0.064
	0.39	0.35	0.18	0.12	0.44	0.063
	0.40	0.30	0.20	0.06	0.42	0.066
	0.37	0.36	0.19	0.06	0.45	0.065
	0.44	0.33	0.18	0.10	0.41	0.066
	0.39	0.37	0.21	0.08	0.44	0.071
	0.33	0.40	0.15	0.03	0.46	0.077
	0.41	0.30	0.17	0.13	0.39	0.079
	0.39	0.33	0.15	0.06	0.48	0.065
	0.39	0.40	0.20	0.07	0.43	0.069
					0.43	0.068
					0.48	0.063
Ave., %	0.40	0.34	0.18	0.08	0.43	0.068
Range, %	0.33-.44	0.28-.40	0.15-.21	0.03-.13	0.39-.48	0.063-.079
S.D.	0.03	0.04	0.02	0.03	0.03	0.005

SECTION XII

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